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Characterization of Intrinsic PAH Biodegradation in Groundwater During Tidal Cycles at the Naval Station Norfolk: Interim Report

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From July 1999 through July 2001, NRL sampled a single groundwater monitoring well located at the Naval Station Norfolk over the course of several tidal cycles. Groundwater at the site is impacted by subsurface fuel spill(s). Groundwater was assessed for fuel hydrocarbon concentrations, stable carbon isotope fingerprints, temperature, salinity, total microbial carbon demand, and PAH mineralization rates. The following was discovered: PAH concentrations were negligible in groundwater although alkanes were detected and used for stable carbon isotope analysis. PAH mineralization rates and bacterial productivity suggest active microbial communities degrade fuel hydrocarbons in the subsurface. Because different parameters (salinity, DO) influence phenanthrene and fluoranthene mineralization independently, there may be distinct bacterial assemblages involved in their biodegradation. There was little tidal recharge at the sampled monitoring well. There was no significant delivery of dissolved oxygen from the adjacent river water (groundwater was hypoxic during entire tidal cycle). There were indications that overall bacterial production varied with season. PAH mineralization rates were independent of season. It appears that fuel hydrocarbons are a larger component of microbial carbon diet during cooler seasons.

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EXECUTIVE SUMMARY

From July 1999 through July 2001, NRL sampled a single groundwater monitoring well located at the Naval Station Norfolk over the course of several tidal cycles. Groundwater at the site is impacted by subsurface fuel spill(s). Groundwater was assessed for fuel hydrocarbon concentrations, stable carbon isotope fingerprints, temperature, salinity, total microbial carbon demand and PAH mineralization rates. The following was discovered:

- PAH concentrations were negligible in groundwater although alkanes were detected and used for stable carbon isotope analysis.
- PAH mineralization rates and bacterial productivity suggest active microbial communities degrade fuel hydrocarbons in the subsurface.
- Because different parameters (salinity, DO) influence phenanthrene and fluoranthene mineralization independently, there may be distinct bacterial assemblages involved in their biodegradation.
- There was little tidal recharge at the sampled monitoring well. There was no significant delivery of dissolved oxygen from adjacent river water (groundwater was hypoxic during entire tidal cycle).
- There were indications that overall bacterial production varied with season. PAH mineralization rates were independent of season. It appears that fuel hydrocarbons are a larger component of microbial carbon diet during cooler seasons.

Based on these findings and those of previous reports, we conclude the following:

- The PAH hydrocarbons found in the sediments adjacent to the bulkhead are most likely not from groundwater.
- Degradation rates change rapidly over the course of the tidal cycle. While this may complicate long-term attenuation rate estimates, it does suggest the bacterial assemblage is able to rapidly adapt to changing environmental conditions. This may result in more hydrocarbons being metabolized prior to impact of adjacent ecosystems.
- The relatively closed environment (reduced mixing with estuarine water) and high PAH degradation rates increase the likelihood that intrinsic bioremediation may be used as a remedial alternative once free product is removed.

Future studies should focus on finding locations where there is more tidal mixing of estuarine water into groundwater. At areas where significant tidal exchange occurs, adjacent samples should be sampled and fingerprinted to determining if coupling exists between offsite migration of fuel contaminants and loading to the sediments. In addition studies should be conducted to assess the seasonal variation in contaminant concentrations and biodegradation activity, particularly in winter months. Changes in bacterial assemblage composition may be measured to identify the cause and environmental control of hydrocarbon metabolism in this ecosystem.

CHARACTERIZATION OF INTRINSIC PAH BIODEGRADATION IN GROUNDWATER DURING TIDAL CYCLES AT THE NAVAL STATION NORFOLK

INTERIM REPORT

INTRODUCTION

In groundwater, there is complex cycling of natural organic matter (NOM), nutrients, and metabolites through food webs. Both NOM and contaminant hydrocarbons can be assimilated and respired by bacteria. The contaminant utilization rate is controlled by environmental factors that influence natural carbon and nutrient fluxes through microbial assemblages. Typical site evaluations focus on compound surveys and /or flask biotreatability studies and do not concentrate on determining the factors that control contaminant metabolism. As a result, subsequent remediation strategies may not achieve cleanup goals. Further understanding of the mechanisms controlling contaminant flux and transformation will enable the Department of Defense (DOD) to make scientifically sound and fiscally responsible decisions when choosing bioremediation strategies for site cleanup.

Groundwater flow and tidal energy may have a large impact on the transport and fate of subsurface hydrocarbon contaminants (Khondaker et al. 1997; Marquis and Smith 1994). These factors may increase or decrease the residence time and net transport of dissolved groundwater components. Furthermore, tidal channels and confining qualities of soil environments may have an impact on the amount of subsurface contamination reaching adjacent open waterways (Tang and Jiao 2001; Ataie-Ashtiani et al. 2001; Paul et al. 2000). Not only can tidal fluctuations be responsible for altering the migration rate of contaminants, but may be a vehicle for the flux of nutrients and dissolved oxygen to and from the subsurface (Krest et al. 2000; Ataie-Ashtiani et al. 1999). Generally, the modeling of tidal intrusion processes is performed using the confining qualities of the system being studied. It has been shown that small leaks in confining walls (such as those where piers and bulkheads are located) alter flow characteristics to create "point-source" like discharge (Farrell 1994). By assessing the concentration, movement, and biodegradation of fuel hydrocarbons, we may arrive at a better understanding of how and how much off-site migration occurs.

For many subsurface fuel-contaminated sites, multiple sources exist and complex mixing and transport result in uncertain assessment and organization of remedial action. Stable isotope analyses of elements provides the ability to identify the sources and their fate in complex mixtures of NOM. Isotope analysis of carbon, nitrogen, and sulfur pools has provided a more thorough understanding of organic matter sources and cycling in a variety of ecosystems (Peterson et al. 1994; Fry 1986; Coffin and Cifuentes 1999). Further development of this technology has provided the ability to identify cycling of carbon at a molecular level (Coffin et al. 1990; Silfer et al.1991; Meier-Augenstein 1995; Hullar et al. 1996), allowing identification of specific microbial roles in the biogeochemical cycling of carbon and nitrogen. In addition, stable carbon isotope analysis ($\delta^{13}C$) has assisted in the development and interpretation of bioremediation strategies for ecosystems that are contaminated with organic chemicals (Mueller et al. 1995; Coffin et al. 1997; Aggarwal and Hinchee 1991) The recent development of gas chromatographic (GC) transfer of individual compounds, combusted inline, to an isotope ratio mass spectrometer (IRMS) provides the ability to identify individual contaminant sources (Hammer et al.

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1998). Preliminary experiments demonstrate that the carbon isotope signature in 2-, 3-, 4-, and 5- ring PAHs is stable during vaporization, photolytic decomposition and microbial degradation (O'Malley et al. 1994). Fractionation of carbon isotopes is related to the pool size of the substrate in question. Small pool sizes and anaerobic conditions favor isotope fractionation from source material to bio-products (biomass, CO_2). However, if the source material is the component being analyzed and its pool size is large, no significant fractionation will be observed. If contaminant sources have a broad range in $\delta^{13}C$ in individual hydrocarbon compounds, it is possible to determine the contribution of a single source to the total loading. With $\delta^{13}C$ analysis, the percent of vehicular emissions and crank case oil in the total PAH loading was estimated in the St. John's Harbour, Newfoundland (O'Malley et al. 1996). In a similar study using $\delta^{13}C$ for analysis of benzene, toluene, ethylbenzene, and xylene (BTEX), multiple petroleum sources were shown to be present in groundwater that was thought to be contaminated with one source (Kelley et al. 1997). Other recent research provides further support for the application of carbon isotope analysis to trace the contaminant sources. The approach has been applied in the tracking of nitroaromatic compounds, PCE and TCE (Lollar et al. 2001), and jet fuels (Landmeyer et al. 1996).

Another key step in environmental remediation is confirmation and measurement of contaminant degradation rates. Identification of the role of microorganisms in biogeochemical cycles will assist in monitoring contaminant turnover and development of strategies for site cleanup. Over the past decade basic research in microbial assemblage activities and carbon isotope chemistry has led to development of technology that can identify sources of organic matter cycled through bacteria (Boyd et al. 2001; Coffin et al. 1989; Coffin et al. 1990; Coffin et al. 1994). These techniques have been combined with $\delta^{13}C$ analysis of dissolved and particulate organic carbon (DOC and POC) and dissolved inorganic carbon (DIC) to examine the roles of bacterioplankton in aquatic carbon cycles (Peterson et al. 1994; Coffin et al. 1994; Coffin et al. 1997; Coffin and Cifuentes 1999). The development of these new approaches in isotope biogeochemistry has provided an ability to determine carbon sources that support bacterial production from a variety of anthropogenic, autochthonous, and allochthonous sources. This technology has recently been applied to study petroleum hydrocarbons assimilated by bacteria (Pelz et al. 1998). However, in most terrestrial environments, the $\delta^{13}C$ of anthropogenic carbon sources is nearly identical to natural organic matter, resulting in uncertain identification of sources of carbon cycled through the microbial assemblage (Cifuentes et al. 1996b). Cifuentes et al. (Cifuentes et al. 1996a) used $\delta^{13}C$ of CO_2 to monitor degradation of PAH and BTEX in groundwater and found that results of stable carbon isotope analyses indicated enhanced degradation; however, isotope ratios of the contaminant and indigenous carbon overlapped, thus inhibiting confirmation of contaminant degradation.

A complementary alternate approach for tracing carbon cycling through the microbial assemblage is analysis of natural radioactive carbon. Because petroleum hydrocarbons are aged and contain little radioactive carbon, methods have been developed to use $\Delta^{14}C$ to differentiate between bacterial assimilation of fresh relative to aged carbon (Bauer et al. 1990; Bauer et al. 1992; Bauer et al. 1995; Cherrier et al. 1999). For instance, $\Delta^{14}C$ analysis has been used to study cycling of aged and recent carbon sources in river and estuarine aquatic systems (Schiff et al. 1990). The potential of applying $\Delta^{14}C$ for studying contaminant degradation was demonstrated with CO_2 in the vadose zone of an aquifer that was contaminated with organic solvents (Suchomel et al. 1990). In addition, the combination of $\Delta^{14}C$ and $\delta^{13}C$ provides a two-dimensional tracer technique to analyze the assimilation and respiration of organic matter by bacteria. This approach has been applied to investigate the degradation of petroleum in groundwater with analysis of CO_2 in the vadose zone (Massol-Deyá et al. 1997). $\Delta^{14}C$ ratios were critical in this study because stable carbon isotopes of petroleum hydrocarbon degradation were masked by the metabolic products of methanogenic bacteria. Another application of radiocarbon isotope analysis to survey groundwater contaminant degradation was conducted at a gasoline-contaminated site that was being remediated by an air sparging / soil vapor extraction system (Aelion et al. 1997). At this site, radiocarbon isotope analysis of CO_2 in the vadose zone and DIC in the groundwater showed aerobic petroleum biodegradation was between 59% and 87% of the total CO_2 produced. With a correlation of radiocarbon

isotope ratios between respired CO_2 and bacterial biomarkers, an approach for confirming contaminant degradation is available. These parameters coupled with analysis of bacterial production and individual hydrocarbon mineralization rates provide prediction of the time for natural site remediation.

Significant cost savings will result from implementation of an appropriate cleanup strategy for specific site conditions or from a scientific-based decision to not further impact the area. Additional cost savings could result from data documenting the extent to which the Navy is responsible when negotiating with other potentially responsible parties (PRPs). These savings could result from reduced sampling costs associated with integrated studies or reduced legal liability.

Site Description

Naval Station Norfolk is located on Sewell's Point in Hampton Roads, Virginia. It is the world's largest Naval Station and home port to 78 ships and 133 aircraft. There are 14 piers along its 4-mile waterfront and 15 aircraft hangars as well as other military support facilities on its 3,400-acre area (Fig. 1). Extensive former and current refueling operations have released hydrocarbons into the subsurface environment in several locations on the base. Groundwater flow adjacent to fuel plumes may leach soluble components and serve to carry contaminants downgradient; in this case, towards the Elizabeth River and Chesapeake Bay. Near the pier areas, a bulkhead separates the shoreside from the river and Bay. Fueling operations around the northwestern end of the Base have released Bunker C and other fuels into the subsurface. A network of monitoring wells has been installed to determine the extent and distribution of fuel contamination in the subsurface. This study was conducted at a groundwater well adjacent to Pier 7 at the northwestern end of the Base (Fig. 2). Free product is often found at this well.

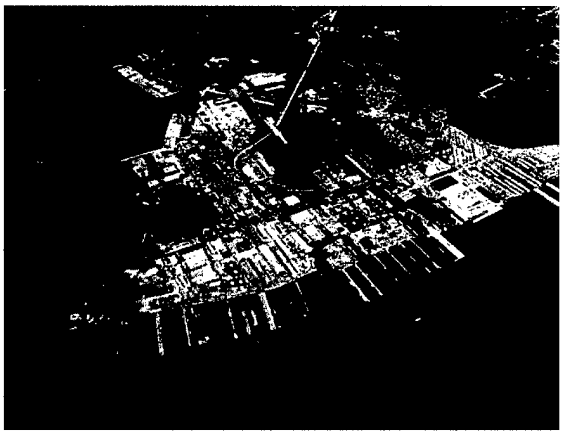

Fig. 1 — Naval Station Norfolk

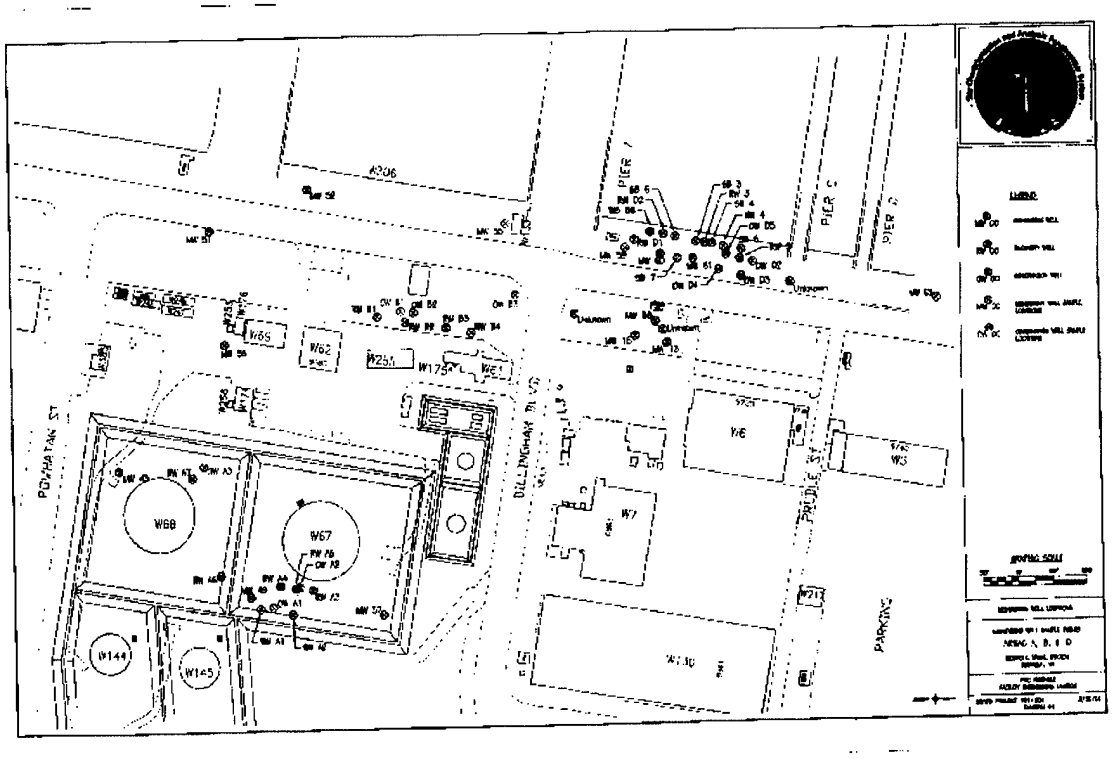

Fig. 2 — Sample site showing MW

Objectives

This study evaluates the effect of tidal cycles on the biodegradation of petroleum hydrocarbons in a groundwater well at the Naval Base Norfolk. PAH and aliphatic hydrocarbon concentrations and stable carbon isotope ratios were determined in groundwater over the course of several tidal cycles. Additionally, contaminant mineralization[1] and bacterial production[2] were measured on the same samples.

Approach

The study of contaminant sources and biodegradative processes involves physical, chemical, and biological characterization of the study site. Data collected to date and in future seasonal samples will be used to determine hydrocarbon source, transport and intrinsic rates of biodegradation occurring in groundwaters adjacent to the Elizabeth River and Chesapeake Bay.

[1] Mineralization is the complete utilization of an organic compound by bacteria. The end product is CO_2.
[2] Bacterial production is the growth rate of the consortium of bacteria in a natural assemblage. The values are often given in units of cells produced per volume per unit time or in terms of the carbon required to produce the cells.

Physical Characterization

Standard water quality surveys were conducted to determine temperature, conductivity, and dissolved oxygen over the course of tidal cycles.

Chemical Characterization

Groundwater was analyzed for concentrations of aliphatic hydrocarbons and PAHs. Stable carbon isotope ratios were determined to delineate sources of hydrocarbons. Relative contributions of different contaminant sources to the total organic carbon pool were measured.

Biological Characterization

Contaminant degradation was measured on site using short-term assays. By relating contaminant concentration and biodegradation to the amount of carbon metabolism (demand[3]), the importance of the contaminant as a carbon source was determined relative to other carbon pools available to the microbial assemblage. These analyses provide information for determining in situ contaminant biodegradation, and give insight into changes in the ecosystem that will result from proactive environmental management. The following work provides the key aspects of information necessary to determine the factors controlling intrinsic biodegradation.

Field Sampling and Sample Analysis

This section describes:

- Sampling objectives and locations
- Sample size and frequency
- Field methods
- Analytical methods

Sampling Objectives and Locations

Sampling objectives were to physically, chemically and biologically characterize groundwaters during daily tidal cycles, maintain proper chain-of-custody and control of samples, and follow QA/QC procedures. Sampling was conducted in July 1999, October 1999, March 2000, September 2000, and July 2001. The most comprehensive sampling event was conducted during July 2001. MW-61, located at 4090354.876N × 381770.057E, was sampled repeatedly over the course of several tidal cycles and several dates.

Sample Size and Frequency

Sample size is dependent on the type of sample analysis to be conducted. Groundwater samples were taken in acid-cleaned 500 mL amber glass bottles with Teflon®-lined closures. Samples for chemical analysis were adjusted to ~pH 10 with several pellets of NaOH and stored at 4 °C until extracted and analyzed. Water samples used for biological analysis were immediately transferred to shoreside laboratory facilities for processing within minutes of collection. Sediment samples for PAH and biological analyses were transferred to 50-mL centrifuge tubes. Subsamples were immediately removed for mineralization and production assays. The remaining sediments were stored at 4 °C until processed back at NRL. Sampling matrices are presented in Tables 1 through 5.

[3] The amount of carbon required to support the measured rate of bacterial growth.

Table 1 — Summary of Sample Collection, July 1999

Sampling Event	Sample Type	Analytical Parameter	Number of Field Samples and QC				Total Samples
			Field	Field Blanks	Trip Blanks	Total	
Tidal	Water	PAH	6	1	0	7	7
Cycle		Stable Carbon isotopes	0	0	0	7	7
		Bacterial production	6	1	0	7	7
		Hydrocarb. Mineraliz.	6	1	0	7	7

Table 2 — Summary of Sample Collection, October 1999

Sampling Event	Sample Type	Analytical Parameter	Number of Field Samples and QC				Total Samples
			Field	Field Blanks	Trip Blanks	Total	
Tidal	Water	PAH	12	1	1	13	13
Cycle		Stable Carbon isotopes	0	0	0	0	0
		Bacterial production	12	1	1	13	13
		Hydrocarb. Mineraliz.	12	1	1	13	13

Table 3 — Summary of Sample Collection, March 2000

Sampling Event	Sample Type	Analytical Parameter	Number of Field Samples and QC				Total Samples
			Field	Field Blanks	Trip Blanks	Total	
Tidal	Water	PAH	6	1	0	7	7
Cycle		Stable Carbon isotopes	0	0	0	0	0
		Bacterial production	6	1	1	7	7
		Hydrocarb. Mineraliz.	6	1	0	7	7

Table 4 — Summary of Sample Collection, September 2000

Sampling Event	Sample Type	Analytical Parameter	Number of Field Samples and QC				Total Samples
			Field	Field Blanks	Trip Blanks	Total	
Tidal	Water	PAH	5	1	0	6	6
Cycle		Stable Carbon isotopes	0	0	0	0	6
		Bacterial production	5	1	0	6	7
		Hydrocarb. Mineraliz.	5	1	0	6	7

Table 5 — Summary of Sample Collection, July 2001

Sampling Event	Sample Type	Analytical Parameter	Number of Field Samples and QC				Total Samples
			Field	Field Blanks	Trip Blanks	Total	
Tidal	Water	PAH	12	1	1	14	14
Cycle		Stable Carbon isotopes	12	1	1	14	14
		Bacterial production	12	1	1	14	14
		Hydrocarb. Mineraliz.	12	1	1	14	14
	Sediment	PAH	3	1	1	5	5
		Bacterial production	3	1	1	5	5
		Hydrocarb. Mineraliz.	3	1	1	5	5

Field Methods

This section describes the procedures for collection and preservation of samples.

Sample Collection

A peristaltic pump was used to pull in-well waters to the surface and into collection vessels. Aged Pharmed® tubing was used during all samplings. Approximately three well volumes were purged within 12 hours of sampling. Pier water samples were collected using a weighted bottle suspended with nylon string.

Groundwater Samples

Groundwater samples were collected via peristaltic pump through a Hydrolab DataSonde IV sensor system measuring conductivity, salinity, transmissivity, dissolved oxygen (DO), and density. Samples were taken below the level of free product. The following brief protocol was used: Note time and date in

field log. Collect water sample through peristaltic pump. Note conductivity, salinity, transmissivity, and DO in field log. Transfer water to appropriate sample containers: a) PAH samples to two 500-mL amber bottles, b) microbiological samples to one 20-mL scintillation vial. Preserve PAH samples as appropriate with two pellets of NaOH. Label sample containers and fill in chain-of-custody documents and any other appropriate information in field logbook.

Pier Water Samples

Deploy Hydrolab over pier side. Note conductivity, salinity, transmissivity, and DO in field log. Deploy weighted bottle sampler; collect ~500 mL surface water. Label sample containers, fill in chain-of-custody documents and any other appropriate information in field logbook.

Sediment Samples

Sediments were collected with a 15 × 15 cm petite Ponar grab. The following summarized protocol was used: Note time and date in field log. Deploy sampler to obtain a 15 to 20 cm deep grab sample. Recover dredge. Transfer sediment to appropriate sample containers: a) PAH samples to two 50-mL centrifuge tubes, b) microbiological samples to one 50-mL centrifuge tube. Preserve samples as appropriate by refrigeration or freezing. Label sample containers, fill in chain-of-custody documents and any other appropriate information in field logbook.

Analytical Methods

Analytical methods were chosen for applicability, sensitivity, and conformity. For maximum comparability with existing databases (NPDES discharges, water quality records, etc.), Environmental Protection Agency (EPA) methods (with described modifications) were chosen for most of the chemical analyses. Most microbiological methods were chosen from the scientific literature and have been subject to extensive peer review before being published. There are no standard EPA or compliance analyses that provide the microbiological information sought in this project. The Appendix provides a summary of analytical methods that were used in this project. Analyses are presented in operating procedure format. Data validation, reporting, and quality control are also covered.

RESULTS OF ANALYSES

Physical Analyses

Water Temperature

Groundwater temperature ranged considerably during the October 1999 and July 2001 sampling events with standard deviations greater than 1.0 (Table 6).

Table 6 — Temperature Variation in Groundwater Samples

July 1999	N.D.*	N.D.	N.D.	N.D.
October 1999	21.1	27.6	24.7	2.2
March 2000	17.5	18.1	17.8	0.23
September 2000	23.4	24.9	24.3	0.51
July 2001	22.4	25.1	23.6	1.0

*N.D. Not Determined

Tidal Range

Tidal states were determined from NOAA's validated tidal height database (http://tidesonline.nos.noaa.gov). Chesapeake Bridge Tunnel tidal station was used for all tide estimates. Tides are presented as meters above mean low water level (MLW). Tidal range was highest in October 1999. The range was also wide during September 2000 and July 2001 with considerable standard error (Table 7).

Table 7 — Tidal Heights for Chesapeake Bay Bridge Tidal Station

Sampling Event	Lowest Tidal Level (m)	Highest Tidal Level (m)	Average Tidal Level (m)	Standard Error (±m)
July 1999	0.205	0.622	0.41	0.074
October 1999	0.326	1.14	0.74	0.092
March 2000	0.021	0.82	0.43	0.15
September 2000	0.328	0.957	0.64	0.11
July 2001	0.14	1.06	0.62	0.11

Chemical Analyses

PAHs in Groundwater

PAHs were below minimum confidence detection limits in all groundwater samples taken during all sampling events. However, traces of naphthalene, fluorene, phenanthrene, anthracene, and fluoranthene were observed in a few samples. Many aliphatic hydrocarbons and putative biodegradation products were detected, however they were not quantified because authenticated standards were not concurrently analyzed. More details are presented in the Discussion section.

PAHs in Sediments

Sediment PAH concentrations were relatively high in sediments collected off Pier C during the July 2001 sampling, particularly for some of the lighter molecules (Table 8). Individual PAH concentrations were very similar for those higher in molecular weight than anthracene as standard errors were very low (Table 8). Concentrations of acenaphthene and fluorene were extremely high, indicating petroleum as a candidate source for sediment PAH (c.f. McGroddy and Farrington 1995). However, pyrogenic PAH such as pyrene and fluoranthene were also elevated. More than likely, there are numerous sources for PAH in pier-side sediments, and there is no supporting evidence as to whether they originate from Navy or non-Navy sources.

Table 8 — PAH Concentrations in Pier Sediment Samples

PAH	Pier 1 ($\mu g\ g^{-1}$)	Pier 2 ($\mu g\ g^{-1}$)	Pier 3 ($\mu g\ g^{-1}$)	Average ($\mu g\ g^{-1}$)	Standard Error ($\pm\mu g\ g^{-1}$)
Naphthalene	N.D.	N.D.	N.D.	N.D.	N.D.
Acenaphthalene	10.8	1.26	2.29	4.78	3.02
Acenaphthene	146	8.82	124	92.9	42.5
Fluorene	192	3.15	197	130	63.8
Phenanthrene	5.84	6.25	11.8	6.00	3.30
Anthracene	5.27	5.13	5.09	5.16	0.05
Fluroanthene	13.1	14.8	17.0	14.9	1.13
Pyrene	8.91	9.92	11.7	10.2	0.82
Benzo[a]anthracene	N.D.	N.D.	N.D.	N.D.	N.D.
Chrysene	4.84	3.04	3.29	3.72	0.56
Benzo[b]fluoranthene	1.82	1.45	1.48	1.58	0.12
Benzo[k]fluoranthene	1.76	1.32	1.51	1.53	0.13
Benzo[a]pyrene	0.50	0.45	0.47	0.47	0.01
Indeno[1,2,3-cd]pyrene	0.72	0.54	0.51	0.59	0.07
Dibenzo[a,h]anthracene	1.35	1.12	1.04	1.17	0.09
Benzo[g,h,i]perylene	1.21	0.95	0.87	1.01	0.10

*N.D. Not Detected

Alkane $\delta^{13}C$ Analysis

Alkane $\delta^{13}C$ was analyzed in samples that were prepared for the PAH concentrations. Samples were run on a GC with a split flow that runs individual compounds through an ion trap mass spectrometer for identification and an isotope ratio mass spectrometer for fingerprinting the sources. The GC with an SPB-5 15-m column was ramped to 290 °C over 19 minutes for separation of alkanes from decane to heptadecane (i.e., C10 through C17). Comparisons were made between tanks of new petroleum sources and a ground water sample (Table 9). All values are relative to Pee Dee Belemnite. Instrument precision was ± 0.41 ‰. There was a low variation in C10 through C17 between the four tanks, indicating that all tanks contained the same source of petroleum. For comparison with the alkanes in the groundwater, C13, C15, C16, and C17 analysis indicated that the petroleum contaminant in the ground water is from a different source than the new petroleum stored in the Navy Fuel tanks. These alkanes were consistently 1 to 2 ‰ different in the isotope signatures between samples from the groundwater and the storage tanks. Comparison of older petroleum sources stored in tanks showed a $\delta^{13}C$ with values in the range of the ground waters C14 to C17 alkanes. Additional sampling of the groundwater and possible source tanks will assist in the identification of the spill source(s).

Table 9 — Stable Carbon Isotope Ratios of Alkanes

Hydrocarbon Chain Length	Ground Water	S.D.*	Tank 1	S.D.	Tank 2	S.D.	Tank 3	S.D.	Tank 4	S.D.
C10	-27.15	0.54	-26.45	0.09	-26.83	0.07	-25.87	0.29	-26.24	0.29
C11	-27.11	0.31	-27.14	0.06	-27.34	0.21	-26.52	0.03	-26.75	0.08
C12	-28.77	0.6	-27.14	0.16	-27.73	0.43	-26.79	0.1	-27.59	0.41
C13	-30.00	0.45	-27.52	0.13	-27.60	0.04	-27.16	0.38	-27.38	0.23
C14	-28.64	0.37	-29.38	0.48	-29.31	0.93	-29.40	0.23	-29.79	0.02
C15	-29.14	0.76	-27.19	0.17	-27.38	0.08	-27.53	0.11	-27.34	0.59
C16	-28.69	0.16	-27.96	0.14	-28.58	0.61	-27.87	0.16	-28.28	0.19
C17	-30.55	0.31	-28.81	0.26	-29.30	0.26	-28.64	0.23	-29.04	0.33

*S.D. – Standard Deviation

Dissolved Oxygen

DO saturation ranged from 10.7% to 25.7% in groundwater samples during the July 2001 sampling. DO saturation was statistically higher on average during rising tides than during falling tides (Table 10). DO % saturation was generally low (hypoxic) throughout the sampling event. At other sites, we have noted a decrease in overall bacterial production (carbon utilization) in hypoxic groundwaters relative to high DO groundwaters (Boyd et al. 2001).

Table 10 — DO Concentrations During July 2001 Sampling

Tidal State	Minimum DO Sat (%)	Maximum DO Sat (%)	Average DO % Sat (%)	Standard Error (±%)
Falling	10.7	18.1	13.7	1.2
Rising	13.7	25.7	19.2	1.8

Salinity

Salinity was measured only during the July 2001 sampling event. There was a significant salinity range during the course of the tidal cycle (5.68 to 8.17 ‰) (Table 11). There was a significant difference between the average salinity on the falling tide and rising tide ($P<0.01$). However, this difference was relatively small (0.88 ‰). This may indicate a relatively small tidal intrusion at the monitoring well sampled.

Table 11 — Salinity Ranges During the July 2001 Sampling

Tidal State	Minimum Salinity (‰)	Maximum Salinity (‰)	Average Salinity (‰)	Standard Error (±‰)
Falling	7.03	8.17	7.69	0.22
Rising	5.68	7.70	6.81	0.39

Biological Analyses

Naphthalene Mineralization in Groundwater

Naphthalene mineralization rates ranged from "no detect" to over 7.0 μg L^{-1} d^{-1}. Average mineralization rates were lowest in September 2000 and highest in October 1999 (Table 12). Standard errors were generally over 50% of the mean value, indicating considerable variation in mineralization measurements.

Table 12 — Naphthalene Mineralization Rates

Sampling Event	Minimum Rate (μg L^{-1} d^{-1})	Maximum Rate (μg L^{-1} d^{-1})	Average Rate (μg L^{-1} d^{-1})	Standard Error (±μg L^{-1} d^{-1})
July 1999	0.042	1.31	0.28	0.21
October 1999	0.076	7.00	1.0	0.57
March 2000	0.27	0.69	0.41	0.060
September 2000	N.D.	0.13	0.068	0.018
July 2001	0.006	0.63	0.30	0.18

*N.D. Not Detected

Phenanthrene Mineralization in Groundwater

Phenanthrene mineralization rates ranged from "no detect" to 4.6 μg L^{-1} d^{-1} during July 1999. Average mineralization rates varied considerably through the sampling events with the highest values in July 1999 and July 2001 (Table 13). As with naphthalene mineralization, standard errors were high indicating considerable variation in data from each sampling event.

Table 13 — Phenanthrene Mineralization Rates

Sampling Event	Minimum Rate (μg L^{-1} d^{-1})	Maximum Rate (μg L^{-1} d^{-1})	Average Rate (μg L^{-1} d^{-1})	Standard Error (±μg L^{-1} d^{-1})
July 1999	2.2	4.6	3.5	0.34
October 1999	0.003	0.31	0.093	0.027
March 2000	0.14	0.90	0.45	0.13
September 2000	N.D.	0.12	0.048	0.016
July 2001	N.D.	0.94	0.47	0.34

*N.D. Not Detected

Fluoranthene Mineralization in Groundwater

Fluoranthene mineralization rates were generally lower than naphthalene and phenanthrene mineralization rates for all sampling events. Fluoranthene is primarily considered a pyrogenic PAH, thus is often not found in high concentrations in fuels. The highest fluoranthene mineralization occurred in July 1999 and July 2001 (Table 14). As with the other two PAH growth substrates, average mineralization rates had very high standard errors, indicating considerable variation among the data.

Table 14 — Fluoranthene Mineralization Rates

Sampling Event	Minimum Rate ($\mu g L^{-1} d^{-1}$)*	Maximum Rate ($\mu g L^{-1} d^{-1}$)	Average Rate ($\mu g L^{-1} d^{-1}$)	Standard Error ($\pm \mu g L^{-1} d^{-1}$)
July 1999	N.D.	0.80	0.24	0.12
October 1999	N.D.	0.19	0.063	0.021
March 2000	N.D.	0.008	0.005	0.001
September 2000	N.D.	0.16	0.040	0.027
July 2001	N.D.	0.76	0.14	0.23

*N.D. Not Detected

Bacterial Productivity in Groundwater

Average bacterial production was higher in warmer months (July and September). Production was highest overall in the July samplings (Table 15). Standard errors were not as high as for mineralization, possibly indicating that less variation in production occurred during tidal cycles.

Table 15 — Bacterial Production in Groundwater

Sampling Event	Minimum Rate ($\mu g L^{-1} d^{-1}$)*	Maximum Rate ($\mu g L^{-1} d^{-1}$)	Average Rate ($\mu g L^{-1} d^{-1}$)	Standard Error ($\pm \mu g L^{-1} d^{-1}$)
July 1999	20	62	38	7.0
October 1999	0.18	4.0	1.6	0.39
March 2000	1.3	7.7	4.8	1.0
September 2000	12	37	21	3.6
July 2001	11	56	35	15

*N.D. Not Detected

DISCUSSION

The main goals of this study were to determine the source of groundwater fuel hydrocarbons by chemical fingerprinting and to determine the effects of tidal cycles on their fate and transport. We sampled one monitoring well, adjacent to the Elizabeth River and Chesapeake Bay, repeatedly over the course of several seasons and over tidal cycles. We found variations in physical, chemical and biological parameters. The impact of tidal intrusion on chemical and biological parameters, which has been shown to affect the transport and fate of contaminants and other biogeochemical components (Khondaker et al. 1997; Marquis and Smith 1994), was also evaluated. Nutrients and DO may be transported into (or out of) groundwater during tidal action (Krest et al. 2000; Ataie-Ashtiani et al. 1999). If this occurred during our samplings, we should expect to see correlation between tidal action and concentrations of biogeochemical parameters. In addition, if tidal intrusion played an important role in bringing nutrients and DO to the upland groundwater, we should expect to see seasonal variation in biological activity previously shown (Shiah and Ducklow 1994a; Shiah and Ducklow 1994b) in river waters adjacent to the sampling area.

Seasonal Variation

We noted slight seasonal variation in groundwater temperature and bacterial production (Fig. 3). A relationship between bacterial production and temperature has been demonstrated throughout the Chesapeake Bay open waters (Shiah and Ducklow 1994b; Shiah and Ducklow 1994a; Fisher et al. 1999). If tidal exchange were a major factor affecting microbial activities at the well site, one might expect the same strong correlation between water temperature and production as seen in river waters.

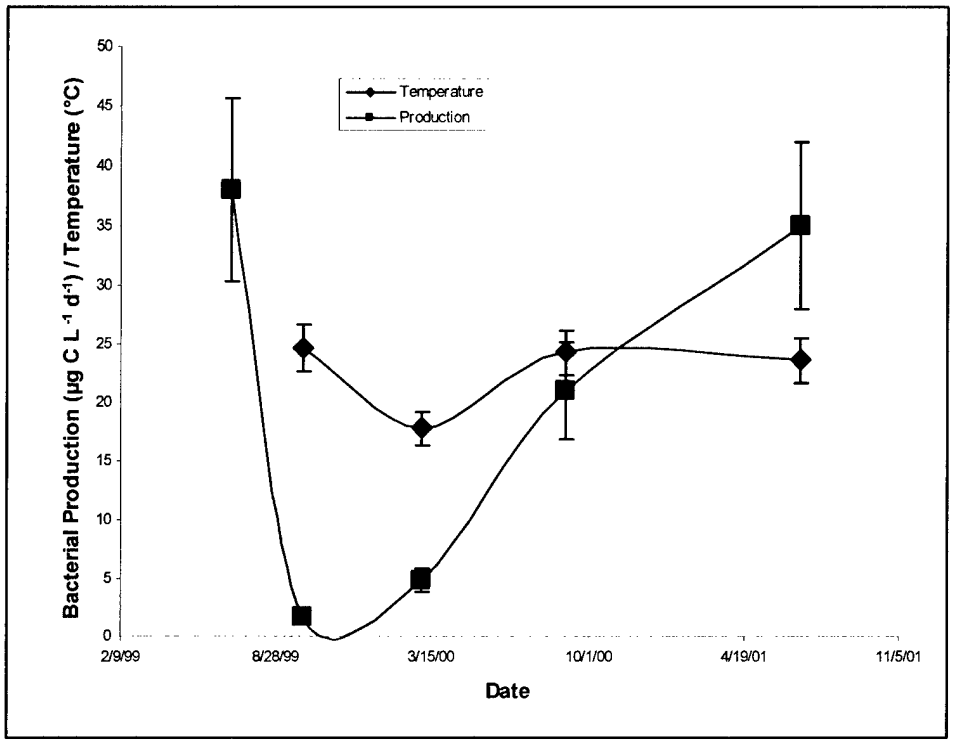

Fig. 3 — Seasonal variation in temperature and bacterial production

There was no correlation between temperature and bacterial production for any of the individual sampling events or for average production over the seasonal samplings ($r^2 < 0.5$). Because there was no large temperature or salinity (see below) swing observed over tidal cycles, it is likely that there was minimal tidal intrusion to the groundwater.

Mineralization of PAHs was chosen as a method to assess in situ hydrocarbon biodegradation because PAHs are a relatively persistent component of fuels. We did not detect appreciable quantities of PAH in any of the groundwater samples. Mineralization showed little to no variation with time of year, although the July samplings had the highest phenanthrene and fluoranthane mineralization rates (Fig. 4). Fluoranthane mineralization rates were considerably lower than the other two substrates. Naphthalene and phenanthrene are found in higher concentrations in fuels than fluoranthane, which is usually associated with pyrogenic processes. There was no correlation between substrate mineralization and temperature for either averaged seasonal or individual tidal cycle sampling (r^2 <0.3). Neither PAH mineralization or bacterial production was significantly affected by temperature changes from the minimal tidal intrusion.

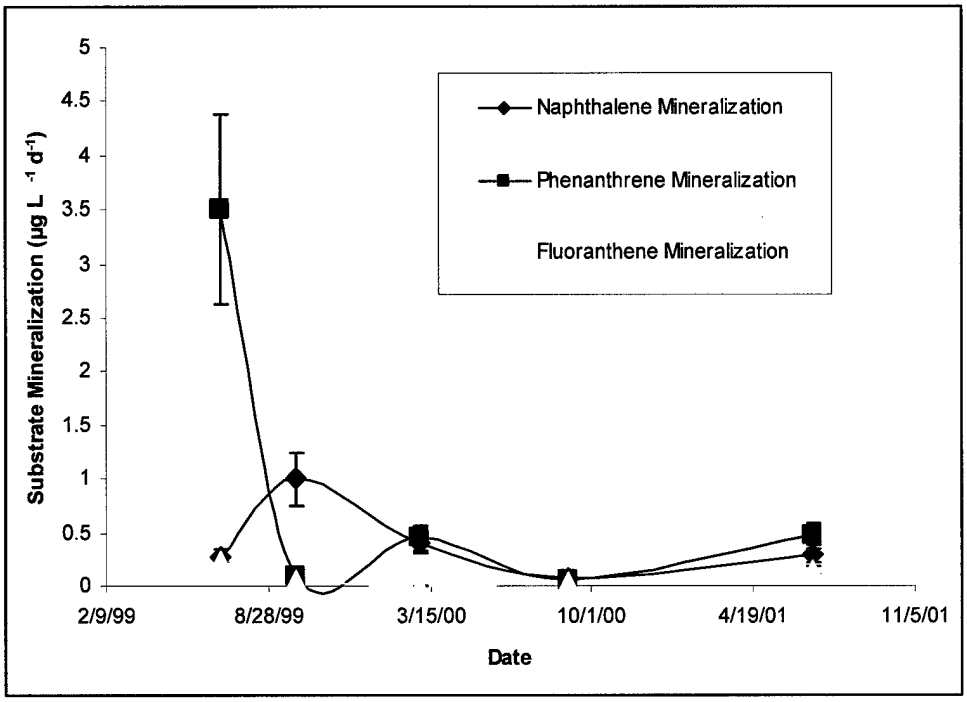

Fig. 4 — Seasonal variation in PAH substrate mineralization

Temperature and Salinity Variation During Tidal Cycles

Both water temperature and salinity represent conservative tracers for determining the impact of tidal recharge within groundwater. We noted a "circular" pattern to both temperature and salinity during our tidal cycle sampling (Figs. 5 and 6). Although the values did not start and end exactly the same, it is obvious that there is mixing of higher temperature, lower salinity groundwater, and lower temperature, higher salinity river water over time in the well. However, the value range (~3 ‰ salinity and ~3 °C) of these parameters indicates relatively weak mixing. We might then expect relatively little impact of recharging nutrients and dissolved oxygen in the groundwater.

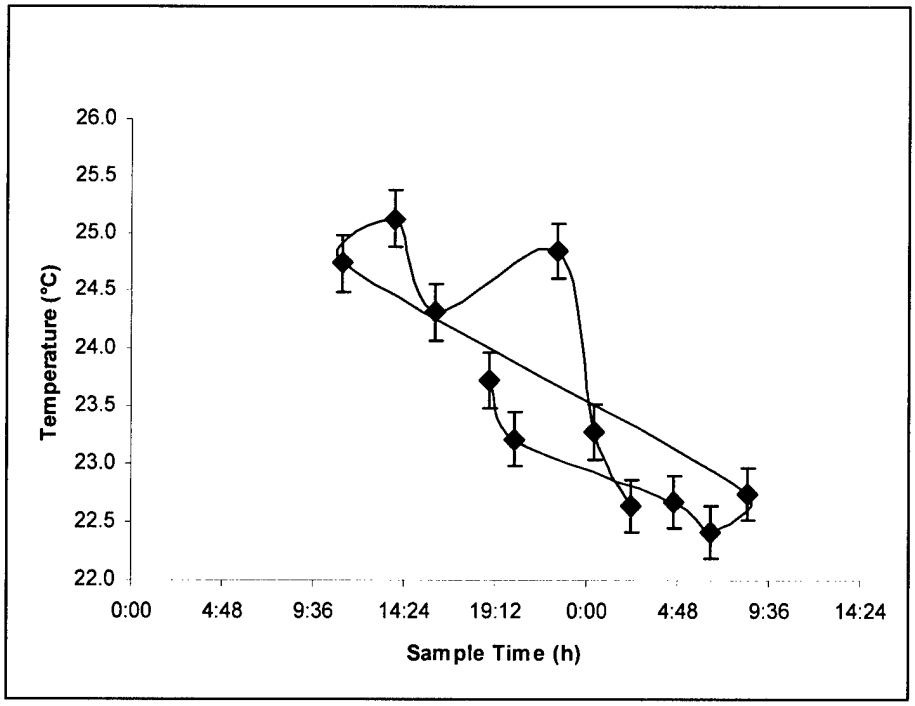

Fig. 5 — Tidal cycle variation in groundwater temperature

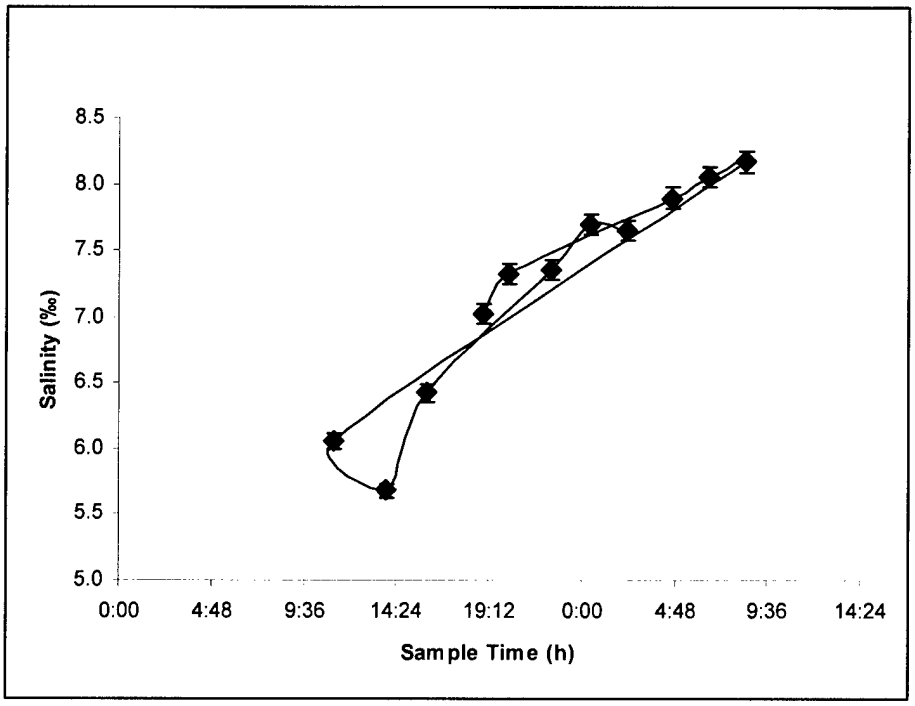

Fig. 6 — Tidal cycle variation in groundwater salinity

DO Variation During Tidal Cycles

Unlike temperature and salinity, DO is a nonconservative tracer because it is actively cycled in biogeochemical reactions. Perhaps, as a result, we found no correlation between DO and tidal height. We also did not observe DO correlation as seen with the conservative tracers, temperature and salinity (Fig. 7.). If DO was not used rapidly, we would expect to see a strong correlation with temperature or salinity which correlated with each other ($r^2 = 0.75$). It is reasonable to assume that DO was subject to utilization during tidal mixing, thus perhaps stimulating biological activity.

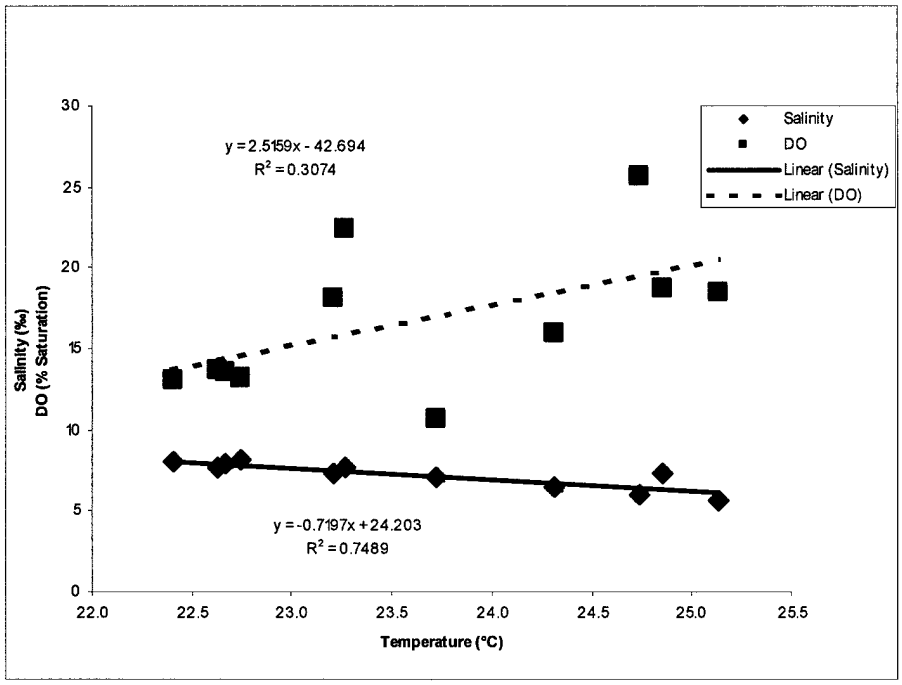

Fig. 7 — Correlation between groundwater temperature and salinity or DO

Napththalene Mineralization During Tidal Cycles

Based on temperature and salinity data we noted only a small degree of tidal recharge in groundwater over the course of our samplings. With influx of river water, we noted changes in DO concentrations that may have stimulated biological activity. Aerobic biodegradation of PAH hydrocarbons are generally thought to occur more rapidly than anaerobic biodegradation (Capone and Bauer 1992). If influx of river water supplied more DO to microbial communities, one would expect to see a rise in the mineralization rates of PAHs, particularly during rising tide. This did not appear to be the case, at least not with any consistent correlation (Figs. 8 to 12). The only consistent result was that the highest naphthalene mineralization occurred during rising tides. Only during the falling tide of the July 1999 sampling was there any correlation between naphthalene mineralization and tidal height ($r^2 = 0.96$). These results indicate there was no "simple" mixing phenomenon driving naphthalene mineralization during tidal cycles.

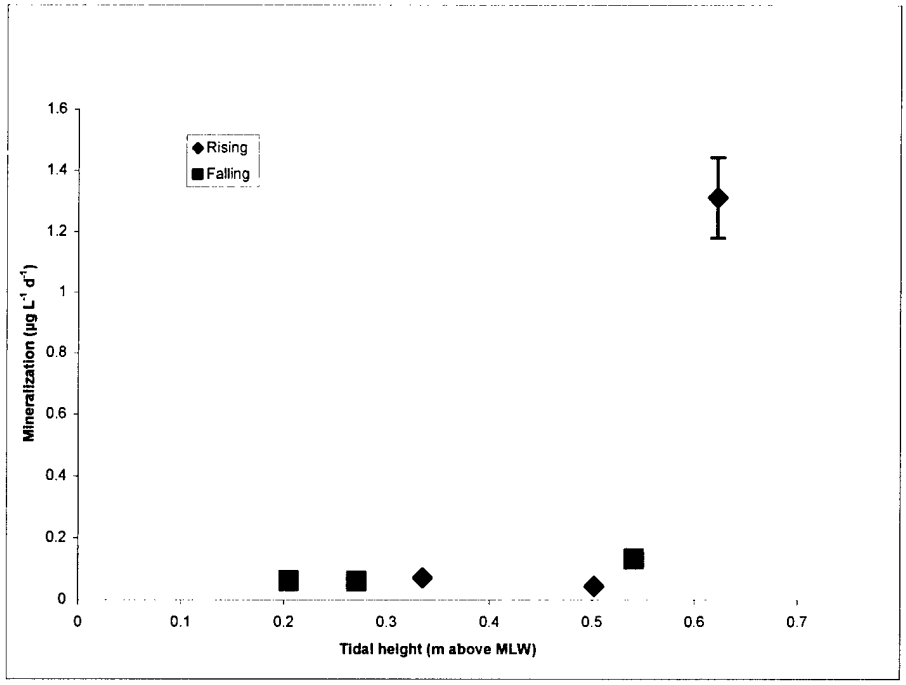

Fig. 8 — Naphthalene mineralization related to tidal height (July 1999)

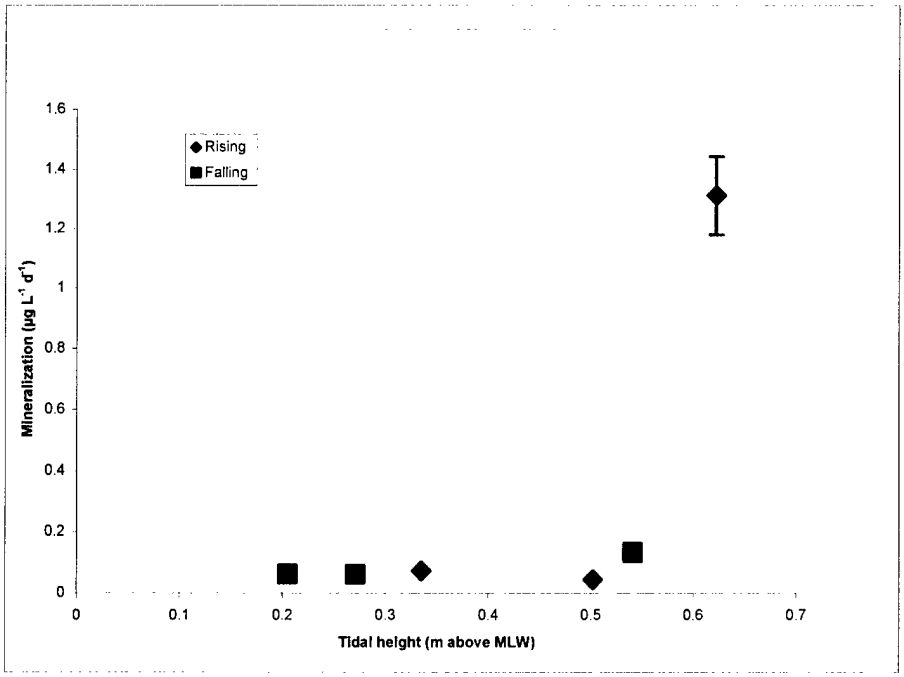

Fig. 9 — Naphthalene mineralization related to tidal height (October 1999)

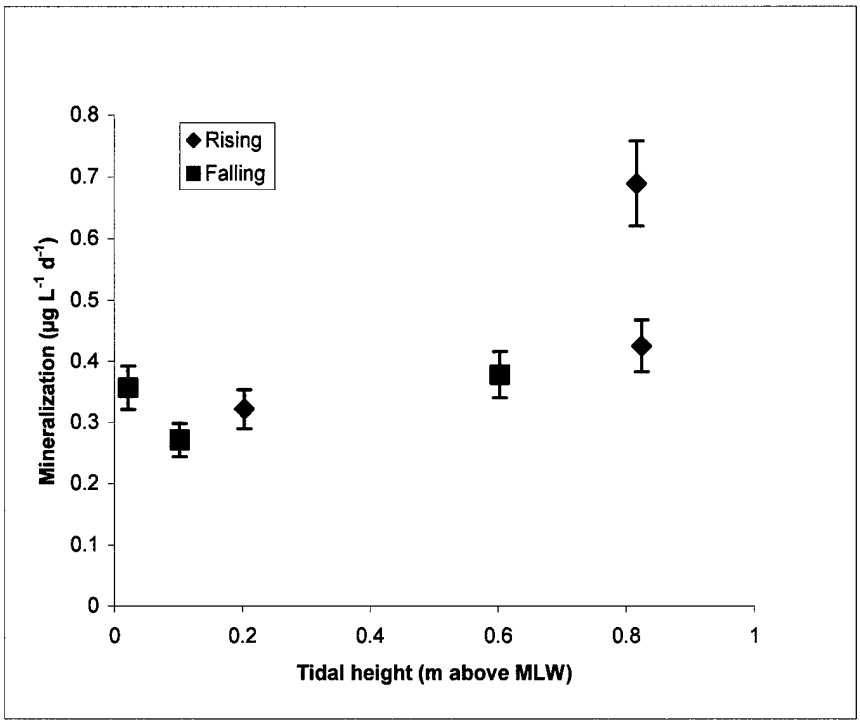

Fig. 10 — Naphthalene mineralization related to tidal height (March 2000)

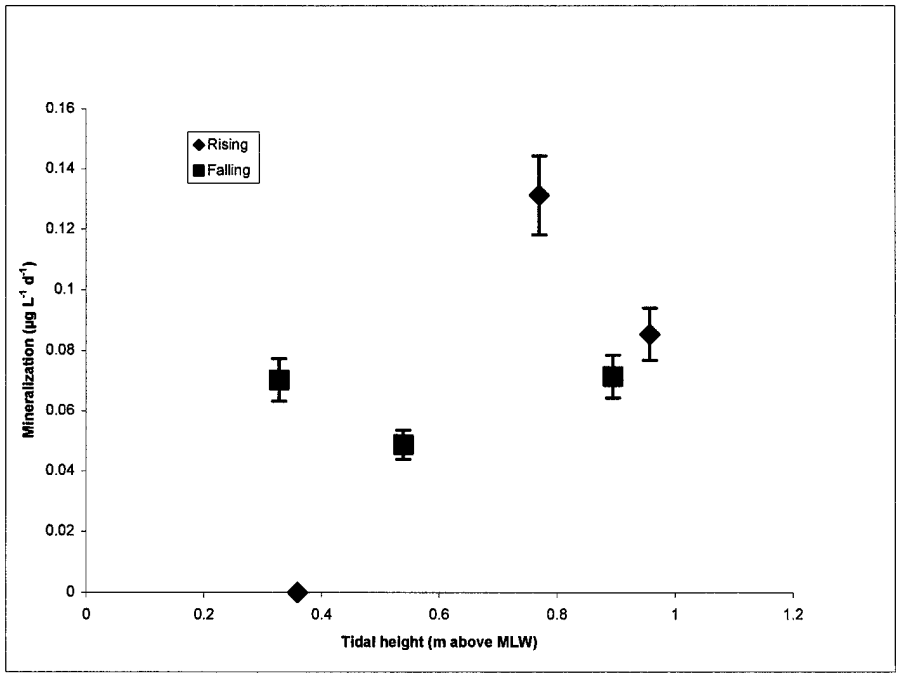

Fig. 11 — Naphthalene mineralization related to tidal height (September 2000)

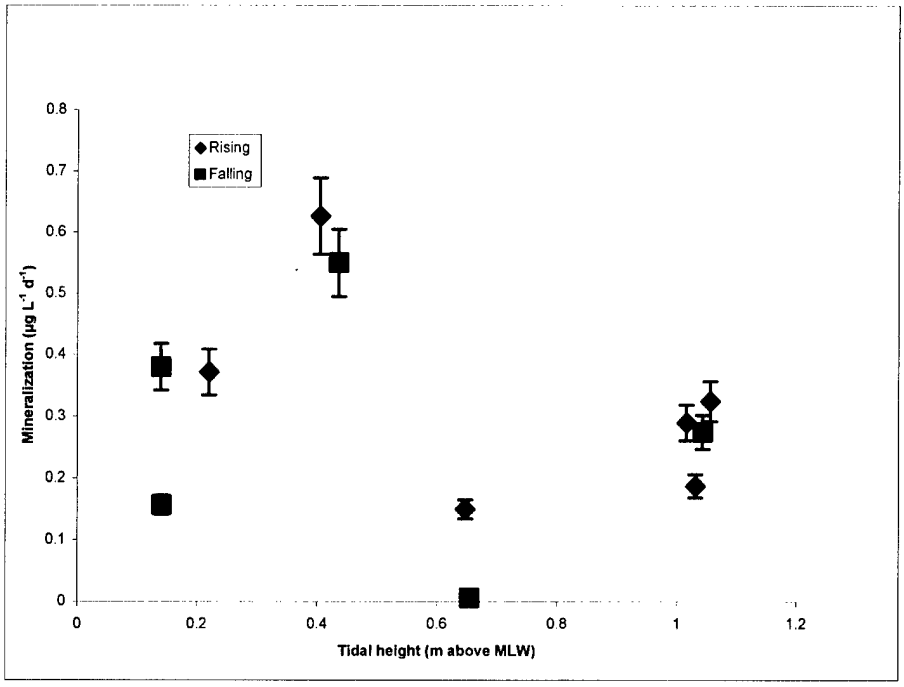

Fig. 12 — Naphthalene mineralization related to tidal height (July 2001)

Another means to determine the effect of DO influx on PAH mineralization is to examine the bacterial growth efficiency (BGE). BGE is calculated by relating the proportion of a specific carbon species respired to CO_2 to the total carbon assimilated by the microbial assemblage. Under aerobic conditions, growth efficiencies are generally higher because molecular oxygen is the most energetically favorable electron acceptor. We noted no discernable trends in BGE when related to tidal height for any of the seasonal samplings (Figs. 13 to 17). It is interesting that BGE is generally increased in the cooler months sampled (October and March). We have noted this phenomenon in other studies. It appears that during cooler months, PAH may become a more important component of the microbial assemblage's carbon demand. It appears in this study that tidal extremes (lowest lows or highest highs) have the highest growth efficiencies. There may be some transient increase in naphthalene degradation at the transition of tidal state.

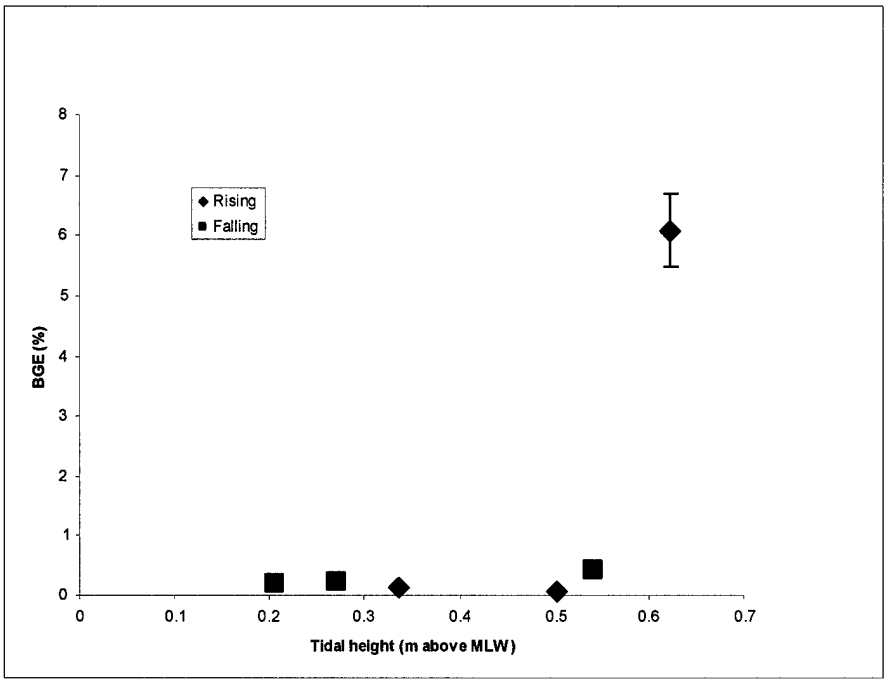

Fig. 13 — Naphthalene BGE related to tidal height (July 1999)

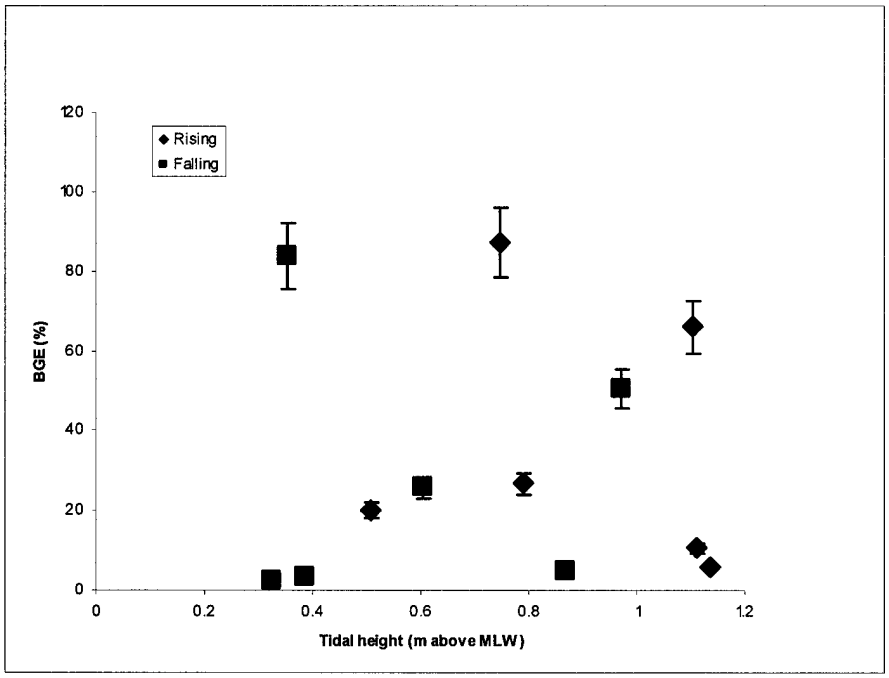

Fig. 14 — Naphthalene BGE related to tidal height (October 1999)

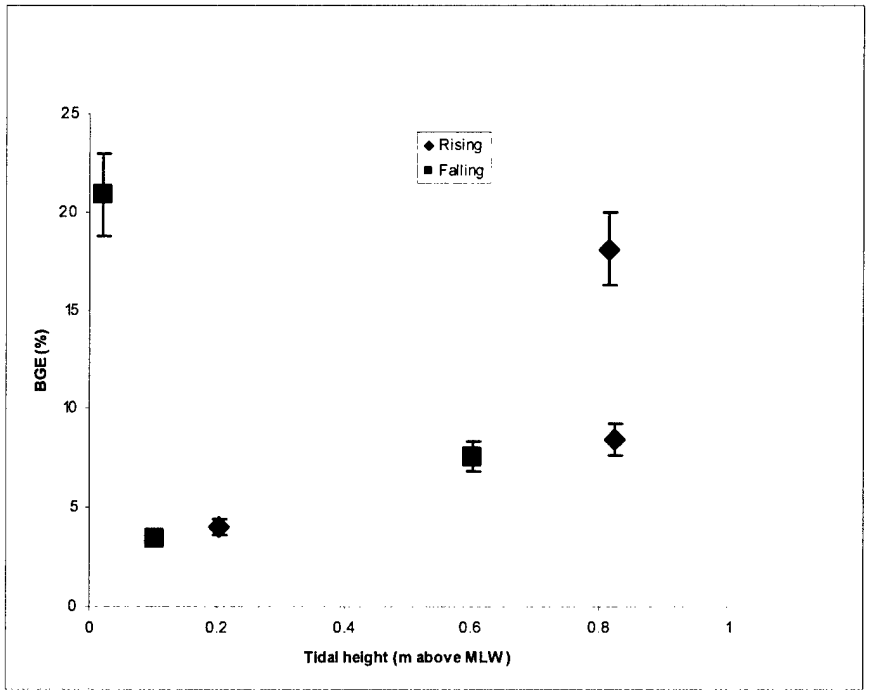

Fig. 15 — Naphthalene BGE related to tidal height (March 2000)

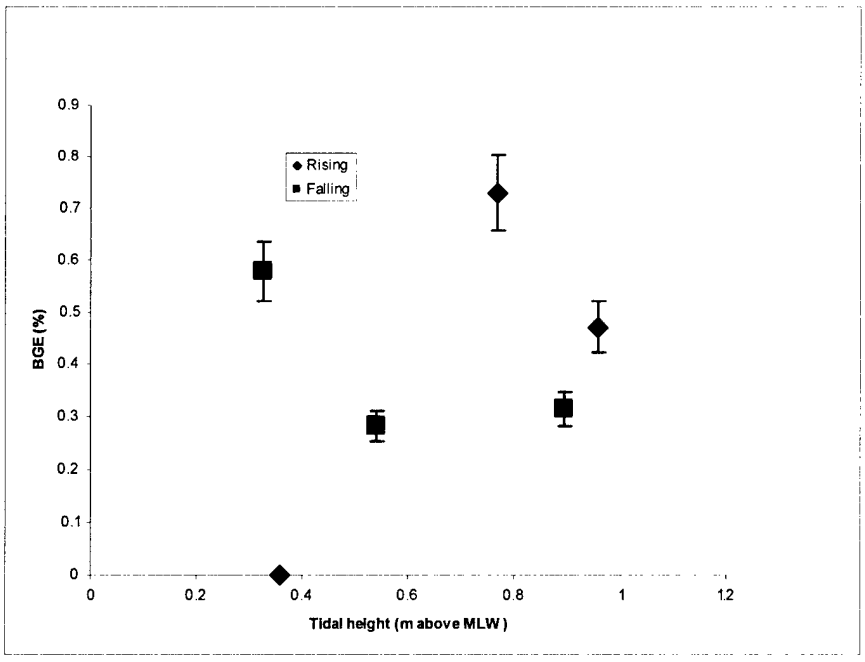

Fig. 16 — Naphthalene BGE related to tidal height (September 2000)

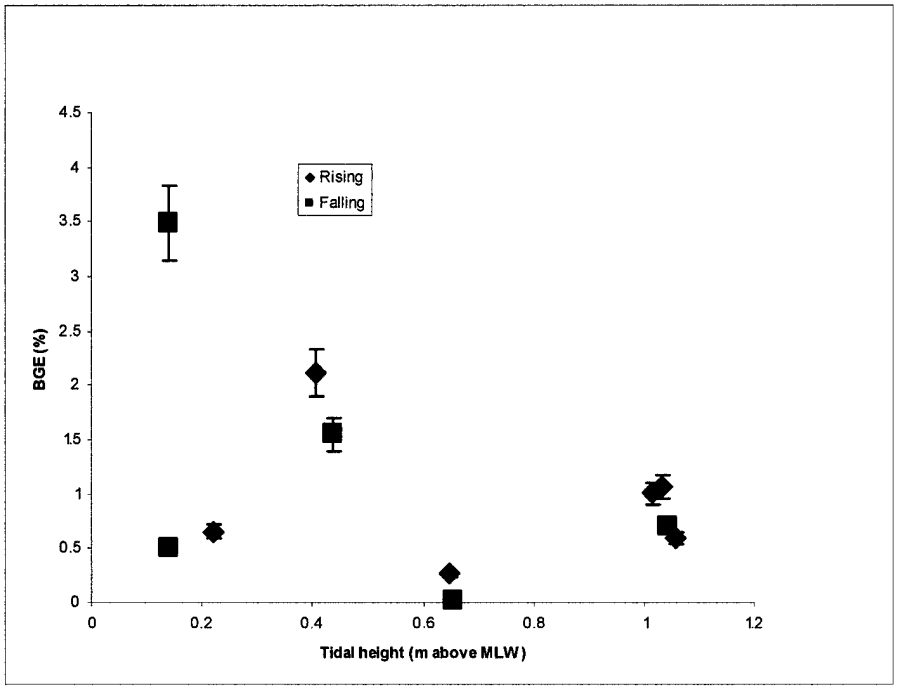

Fig. 17 — Naphthalene BGE related to tidal height (July 2001)

Rates of naphthalene mineralization appeared to covary between samples from the monitoring well and those collected off the pier (Fig. 18). While it is unclear what environmental factors were influencing both environments simultaneously, the rates for both environments did decrease during the 4-hour rain event. Interestingly, bacterial production also covaried between groundwater and pierwater samples over the course of the July 2001 sampling (Fig. 19). In sediment samples from other environments, we have found that naphthalene mineralization and production rates are more tightly coupled that those mineralization rates for phenanthrene and fluoranthene. For instance, we never find sediments with elevated production and low naphthalene mineralization rates. This may be because a greater proportion of the bacterial assemblage can metabolize naphthalene. The environmental factors that are regulating naphthalene mineralization during this sampling may be having a more general influence on bacterial production, which in turn is affecting naphthalene mineralization.

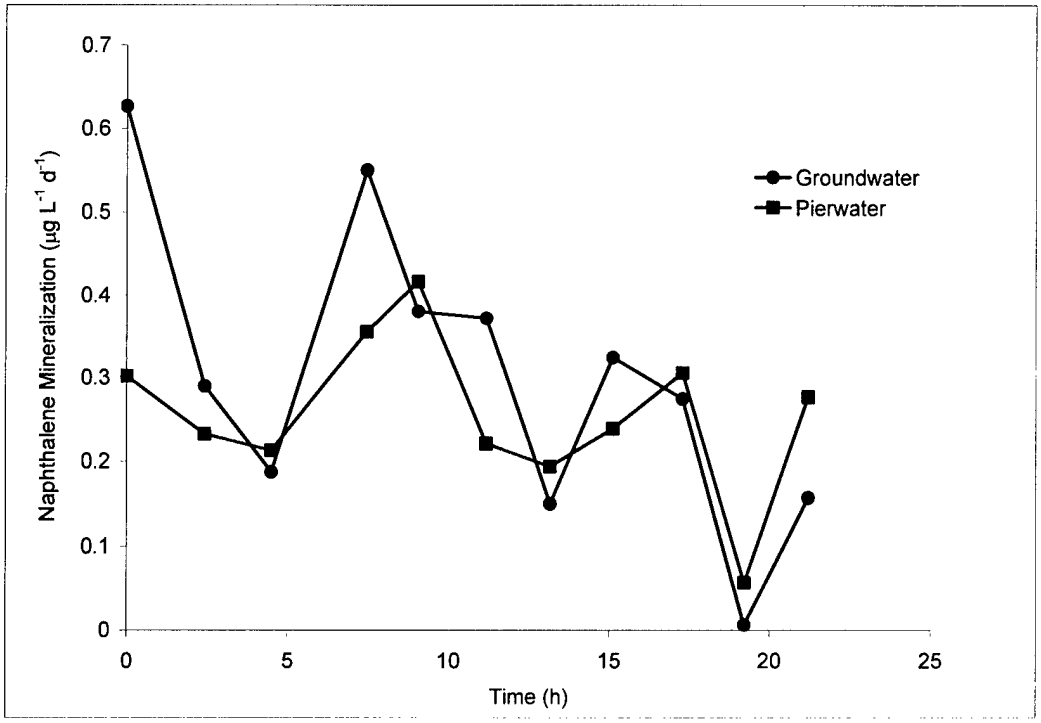

Fig. 18 — Naphthalene mineralization appeared to covary in pierwater and groundwater over the course of the July 2001 sampling

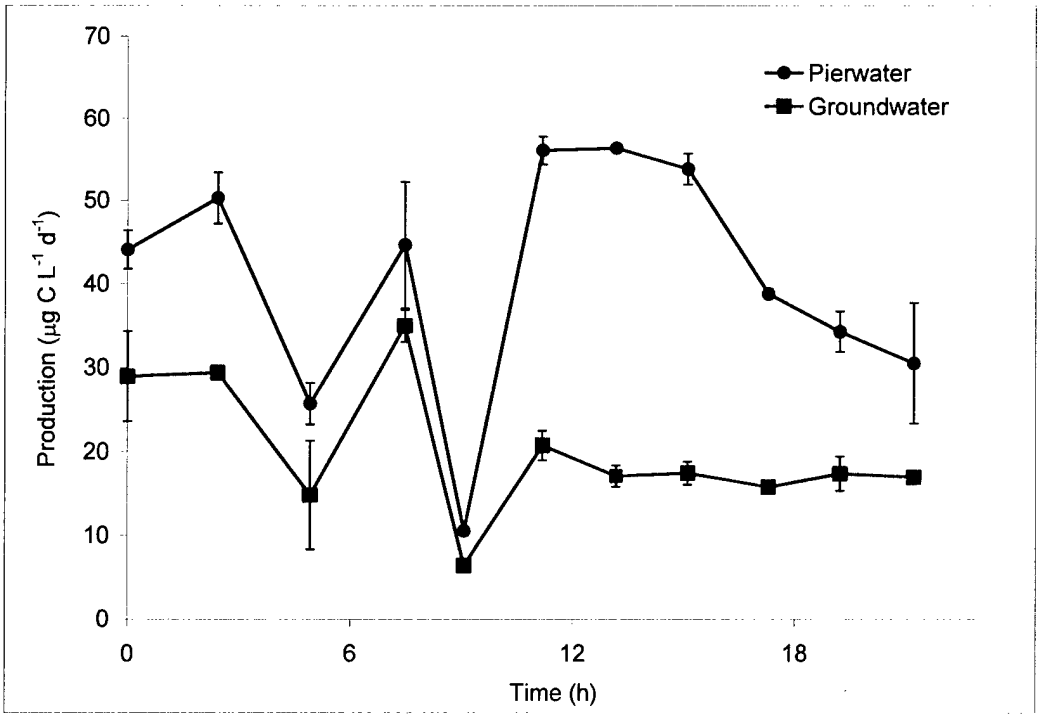

Fig. 19 — In general, bacterial production covaried over the course of the July 2001 sampling with pierwater values always exceeding those for groundwater

Phenanthrene Mineralization During Tidal Cycles

As with naphthalene mineralization, phenanthrene mineralization showed considerable scatter when related to tidal height for most of the season sampling (Figs. 20 to 24). However, during the March 2000 and September 2000 samplings, there was moderate correlation between phenanthrene mineralization and tidal height. Interestingly, the opposite trends were exhibited. In March 2000, there was an inverse relationship between tidal height and phenanthrene mineralization (r^2>0.76). During September 2000, there was a positive correlation between tidal height and phenanthrene mineralization during the rising and during the falling tide (r^2>0.87). If these data are not separated, there is no significant correlation. In general, it appears phenanthrene mineralization, like naphthalene mineralization was highest at the extremes of the tidal heights.

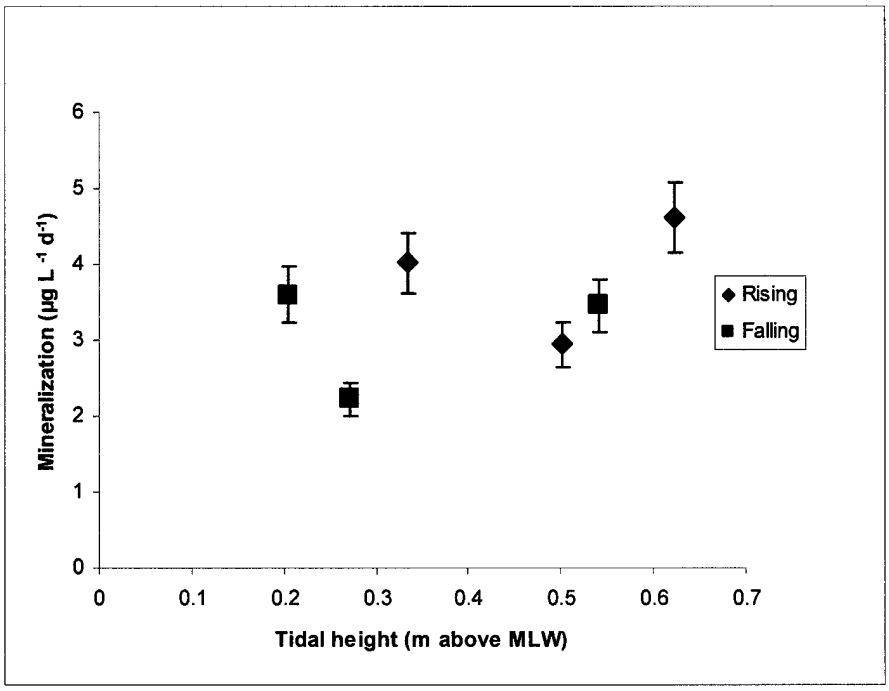

Fig. 20 — Phenanthrene mineralization related to tidal height (July 1999)

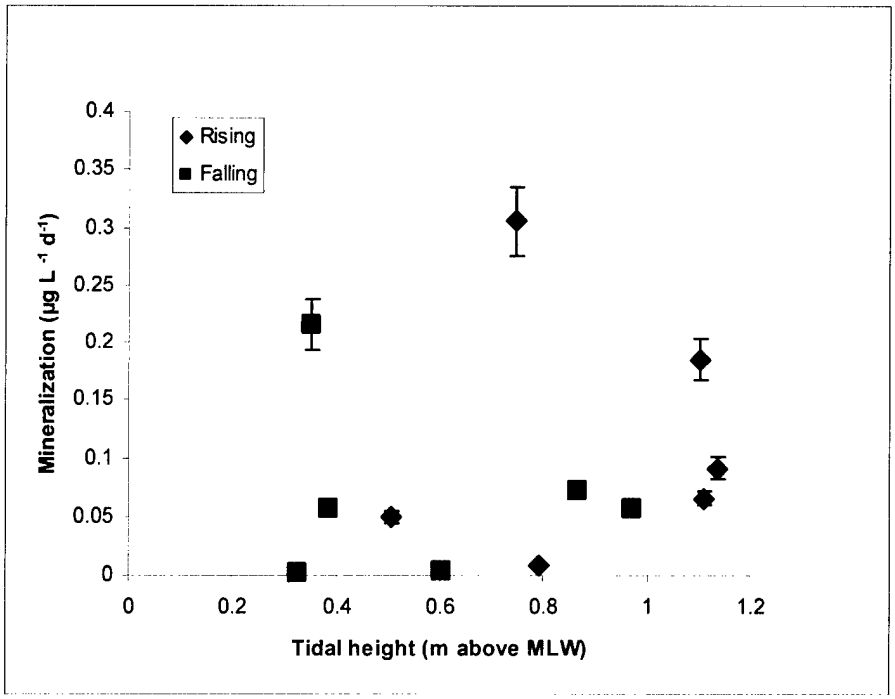

Fig. 21 — Phenanthrene mineralization related to tidal height (October 1999)

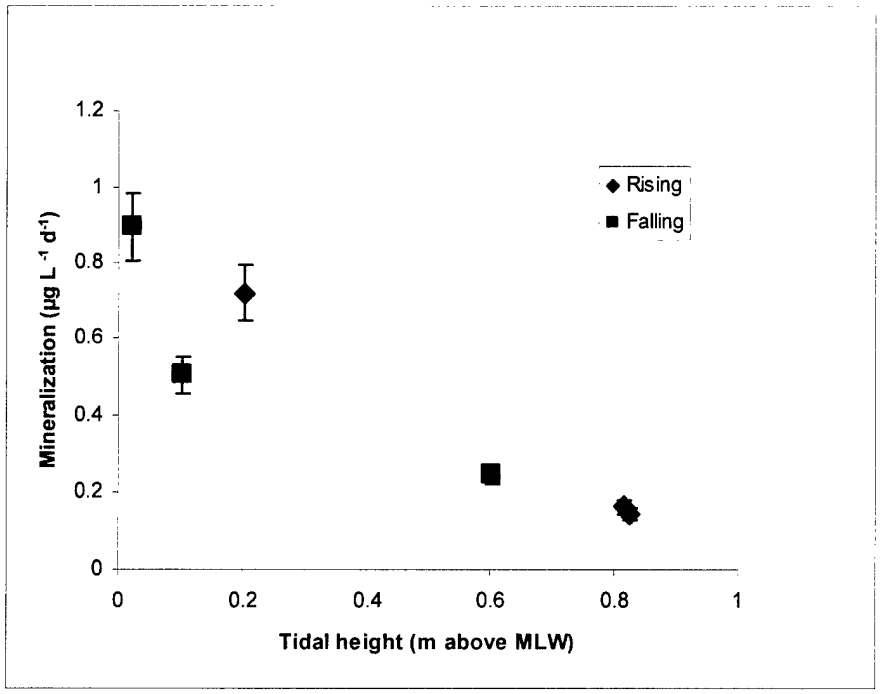

Fig. 22 — Phenanthrene mineralization related to tidal height (March 2000)

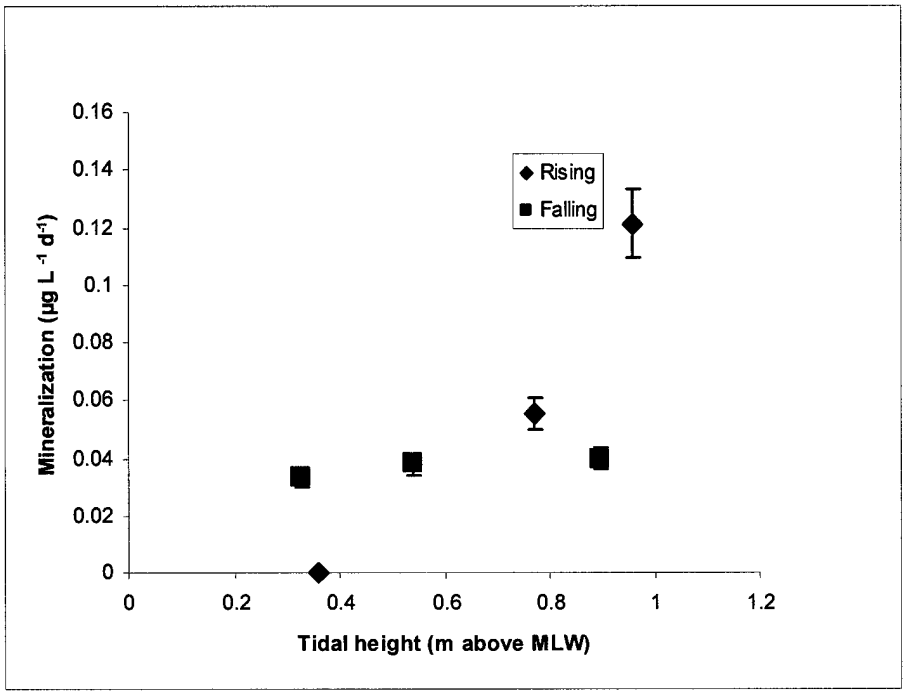

Fig. 23 — Phenanthrene mineralization related to tidal height (September 2000)

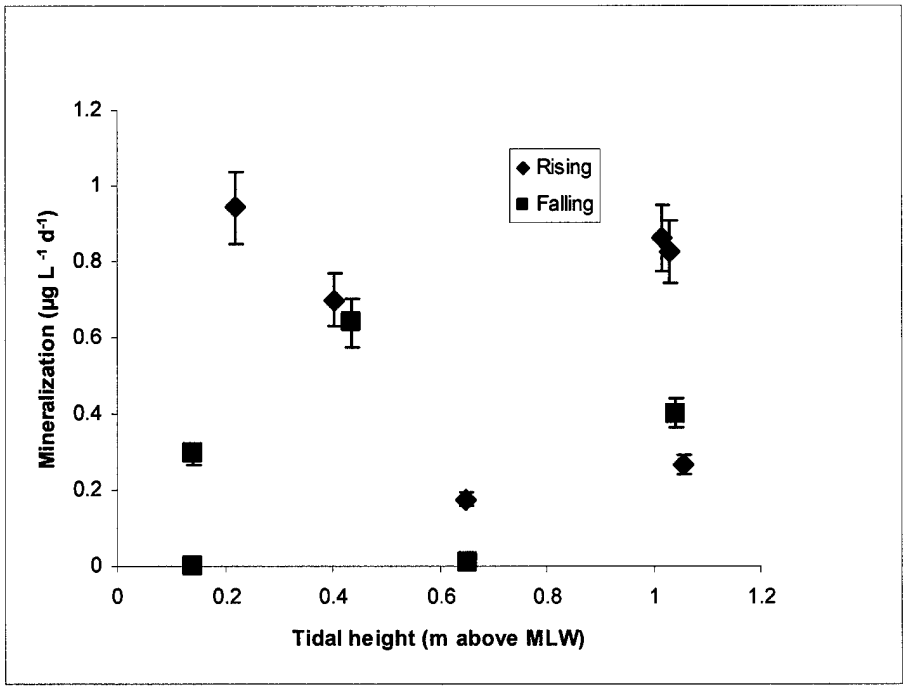

Fig. 24 — Phenanthrene mineralization related to tidal height (July 2001)

As with mineralization, it appears that phenanthrene BGE had the highest levels at the extremes of tidal height (Figs. 25 to 29). Also, as with naphthalene, phenanthrene BGE was highest during cooler months (October and March). This is generally a function of PAH mineralization not being as sensitive to seasonal temperature as bacterial production. This phenomenon has been demonstrated with pelagic bacteria in Chesapeake Bay (Shiah and Ducklow 1994a, 1994b).

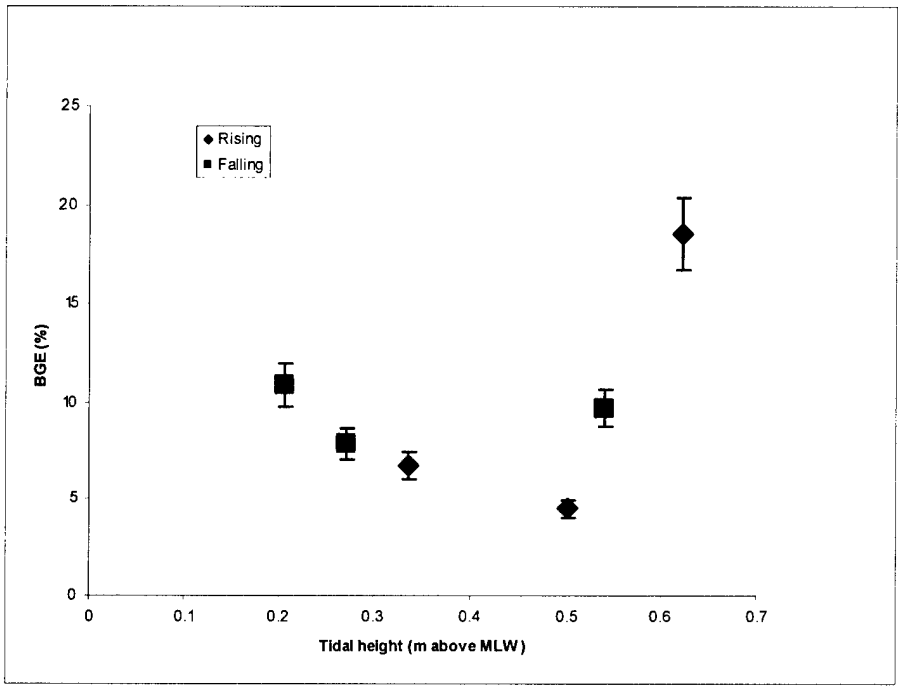

Fig. 25 — Phenanthrene BGE related to tidal height (July 1999)

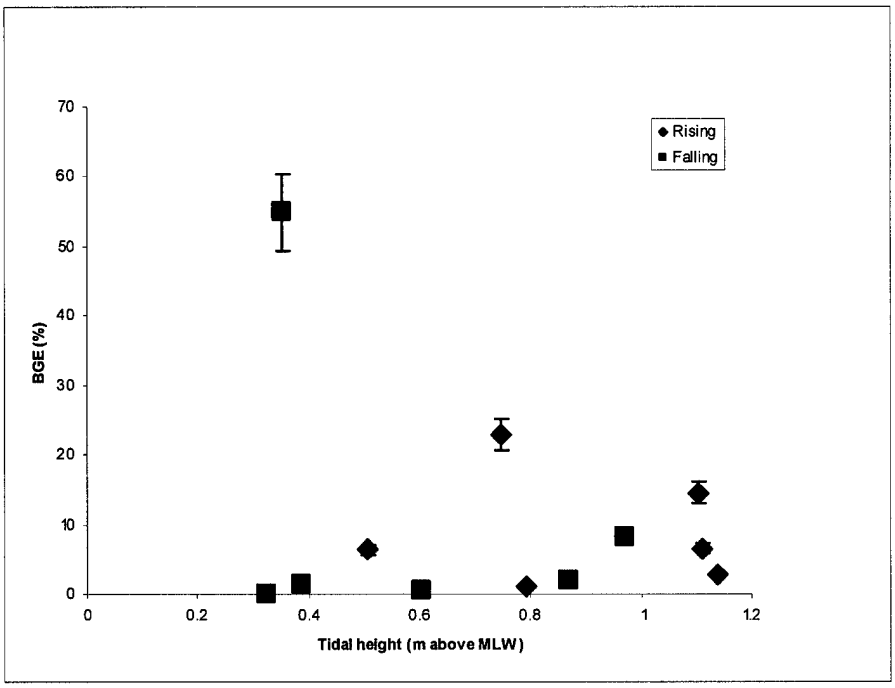

Fig. 26 — Phenanthrene BGE related to tidal height (October 1999)

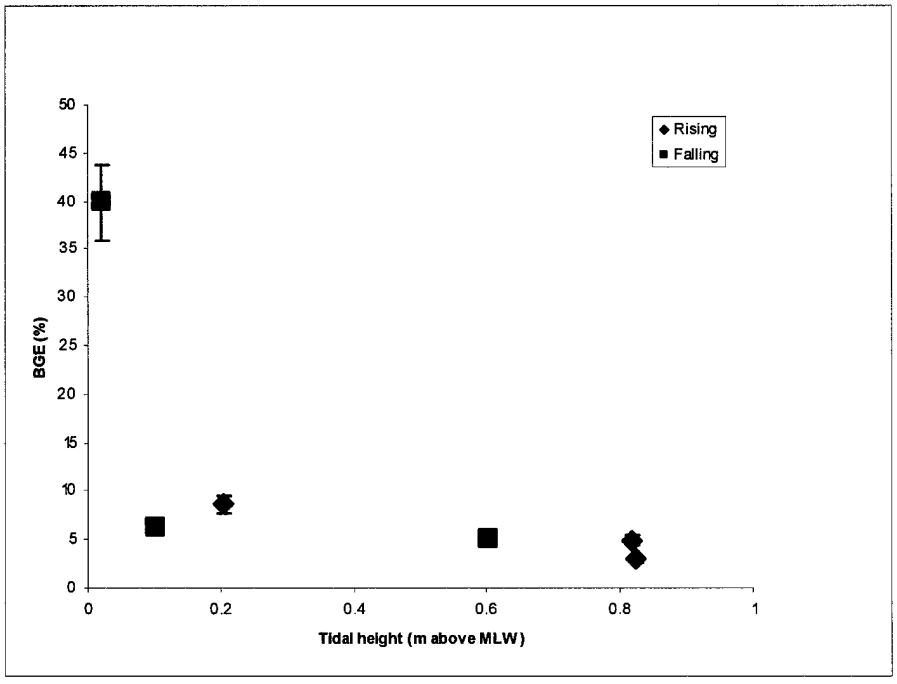

Fig. 27 — Phenanthrene BGE related to tidal height (March 2000)

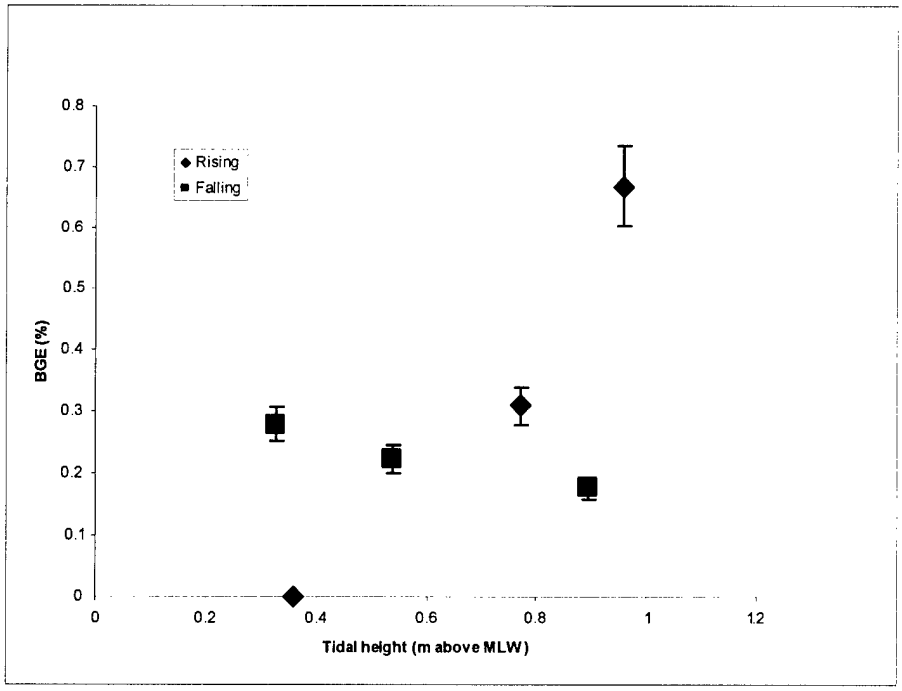

Fig. 28 — Phenanthrene BGE related to tidal height (September 2000)

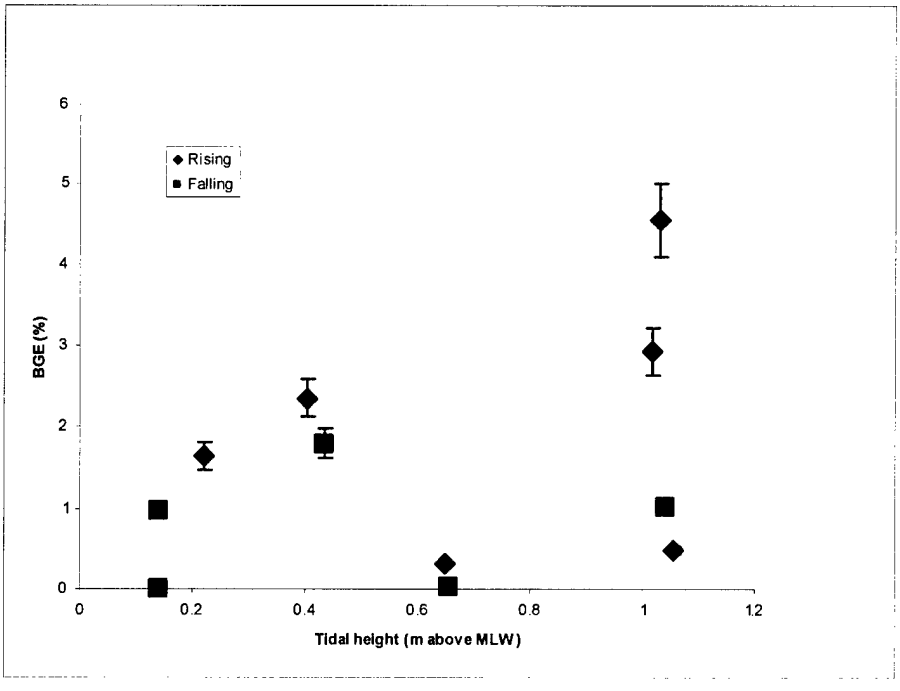

Fig. 29 — Phenanthrene BGE related to tidal height (July 2001)

In general, phenanthrene mineralization was higher in groundwater samples than in pierwater samples though there was a much greater fluctuation in groundwater over the course of the experiment (Fig. 30).

There seemed to be more periodicity to the change in mineralization rates in pierwater than in groundwater. Some of the dramatic changes in the groundwater phenanthrene mineralization may be explained by salinity changes. Mineralization generally decreased with increased salinity though the range of salinity difference was only from 5.5 to 8.2 ‰ (Fig. 31). Others have reported relationships between phenanthrene mineralization rates in surface sediments and salinity (Shiaris 1989).

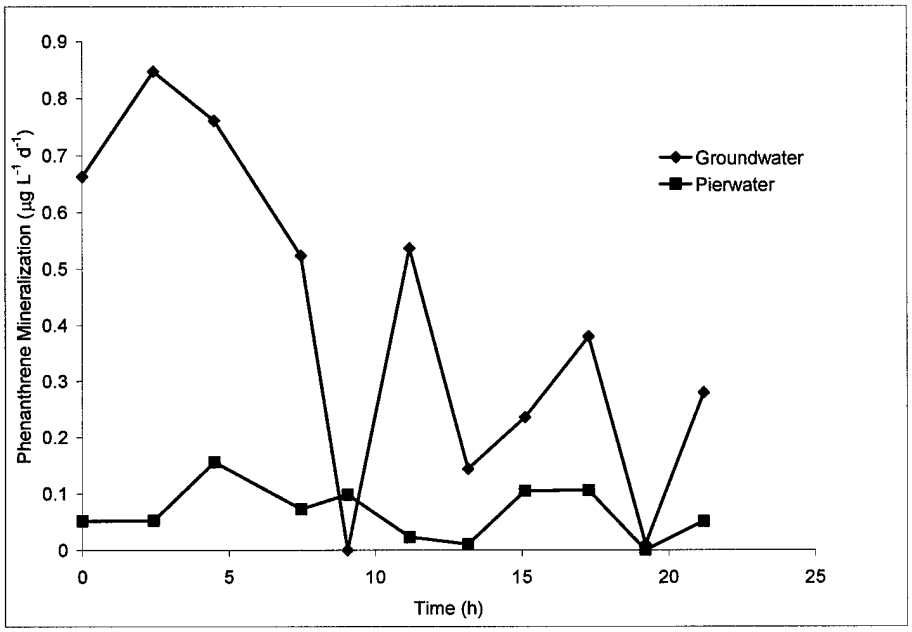

Fig. 30 — Phenanthrene mineralization was often higher in the groundwater than in pierwater from near the bulkhead adjacent to the monitoring well

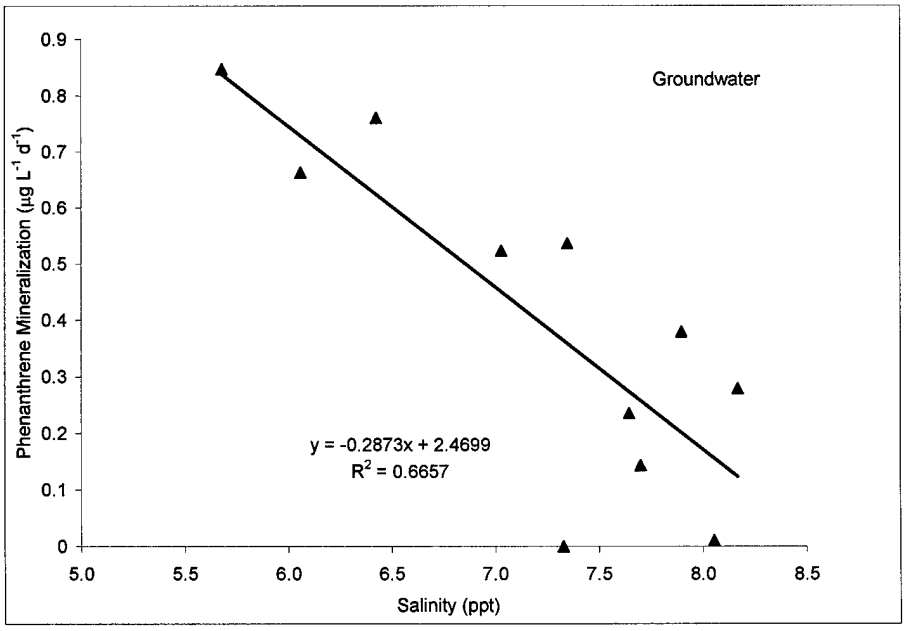

Fig. 31 — Phenanthrene mineralization generally decreased with increasing salinity over the course of the experiment

Fluoranthene Mineralization During Tidal Cycles

Fluoranthene mineralization showed similar trends to naphthalene and phenanthrene mineralization (Figs. 32 to 36). The absolute levels are lower than those for the other two PAH substrates. In October 1999, there appeared to be higher fluoranthene mineralization rates at mid-tides. In July 2001, most of the higher mineralization rates were found in during the lowest portion of the tidal cycle.

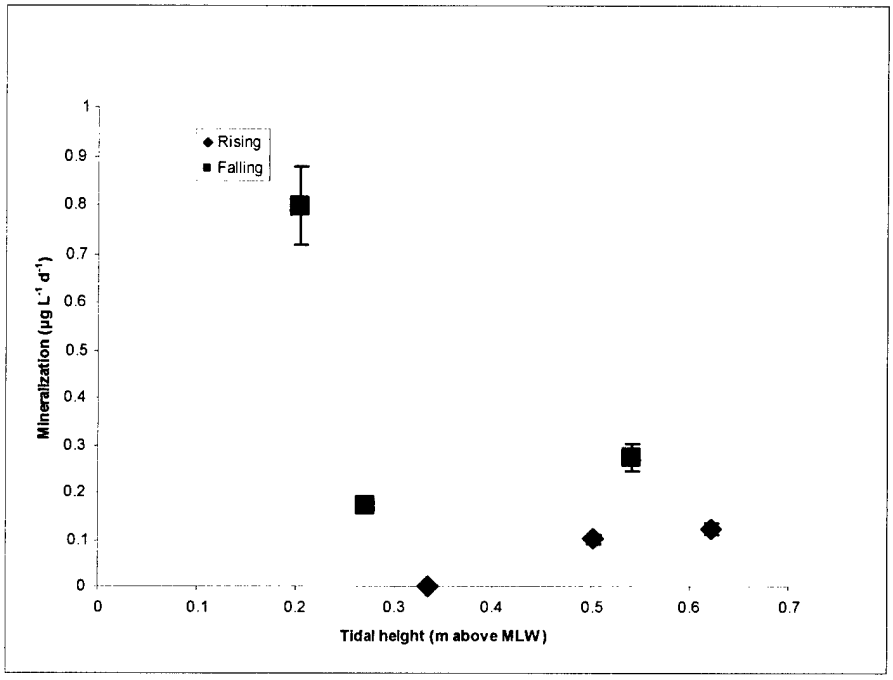

Fig. 32 — Fluoranthene mineralization related to tidal height (July 1999)

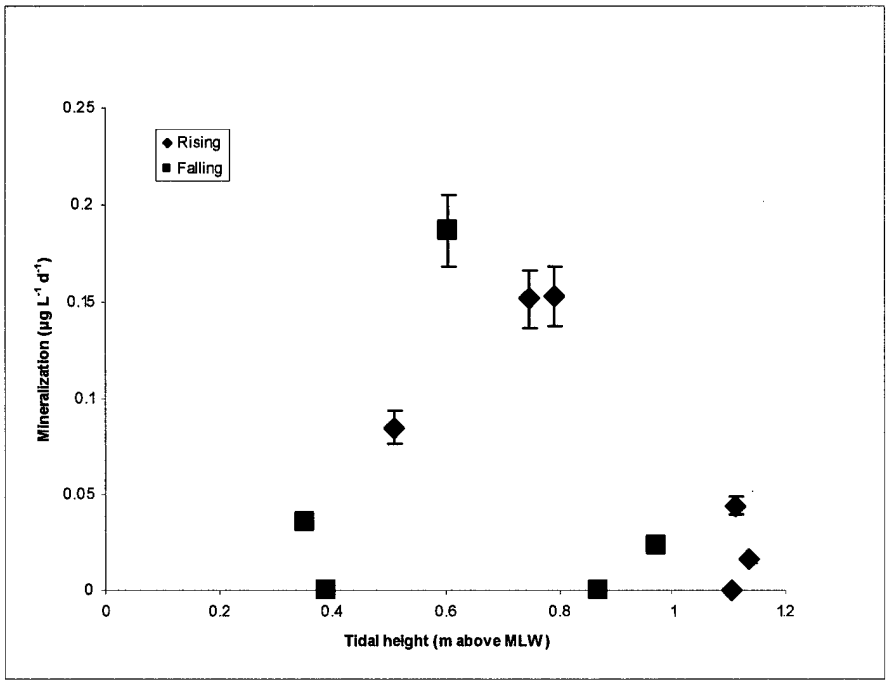

Fig. 33 — Fluoranthene mineralization related to tidal height (October 1999)

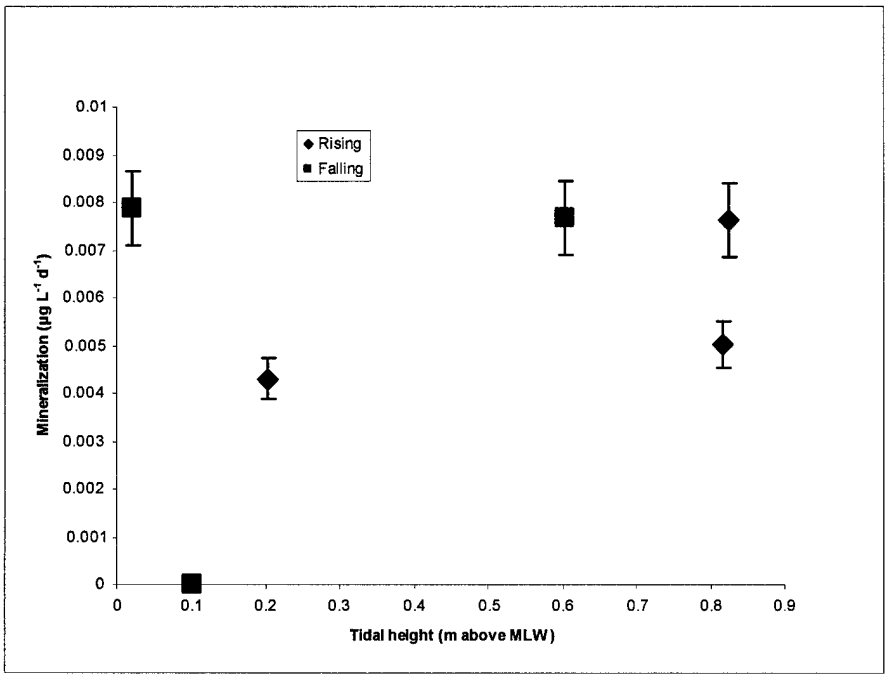

Fig. 34 — Fluoranthene mineralization related to tidal height (March 2000)

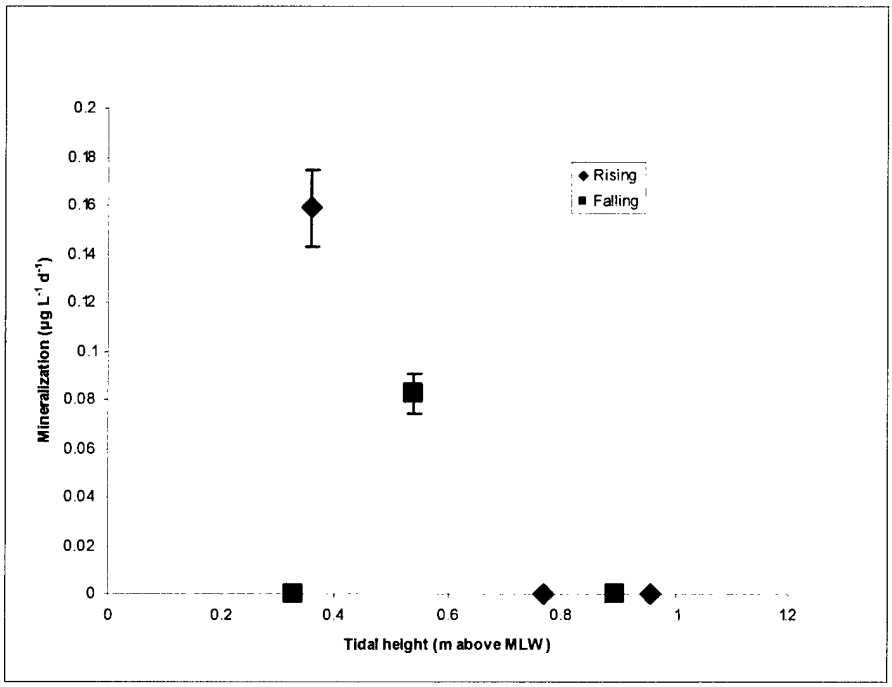

Fig. 35 — Fluoranthene mineralization related to tidal height (September 2000)

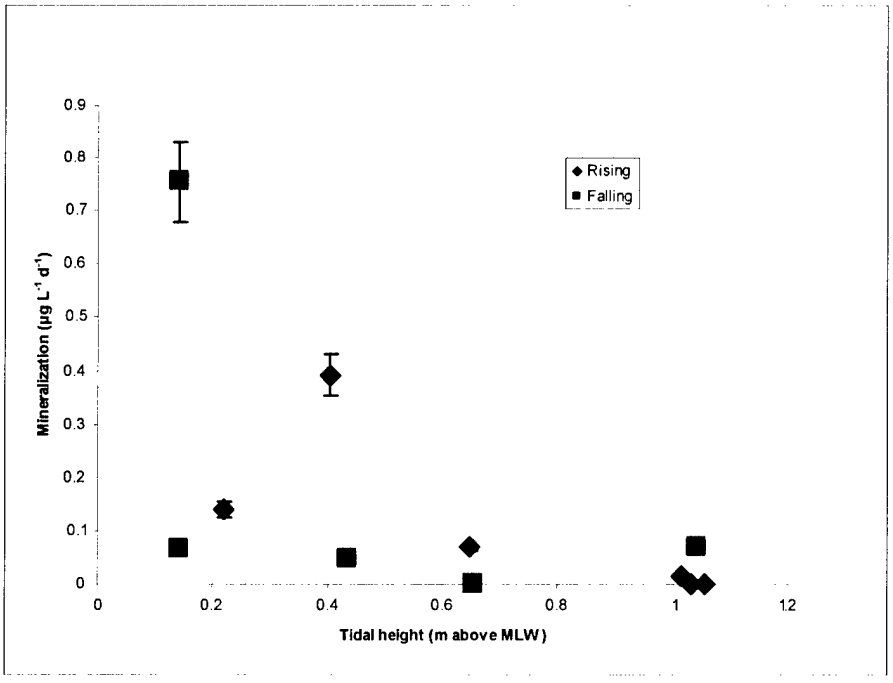

Fig. 36 — Fluoranthene mineralization related to tidal height (July 2001)

Fluoranthene BGE (Figs. 37 through 42) was highest at the lower tides during October 1999 and September 2000 (Figs. 38 and 40). In October 1999, fluoranthene BGE was the highest of any season. All

other seasons had lower than 10% fluoranthene BGE, indicating fluoranthene was a relatively unimportant source of carbon to the bacterial assemblage (Figs. 37, 39, 41, and 42). Again, this should be expected because fluoranthene generally is not a large component of fuel hydrocarbons.

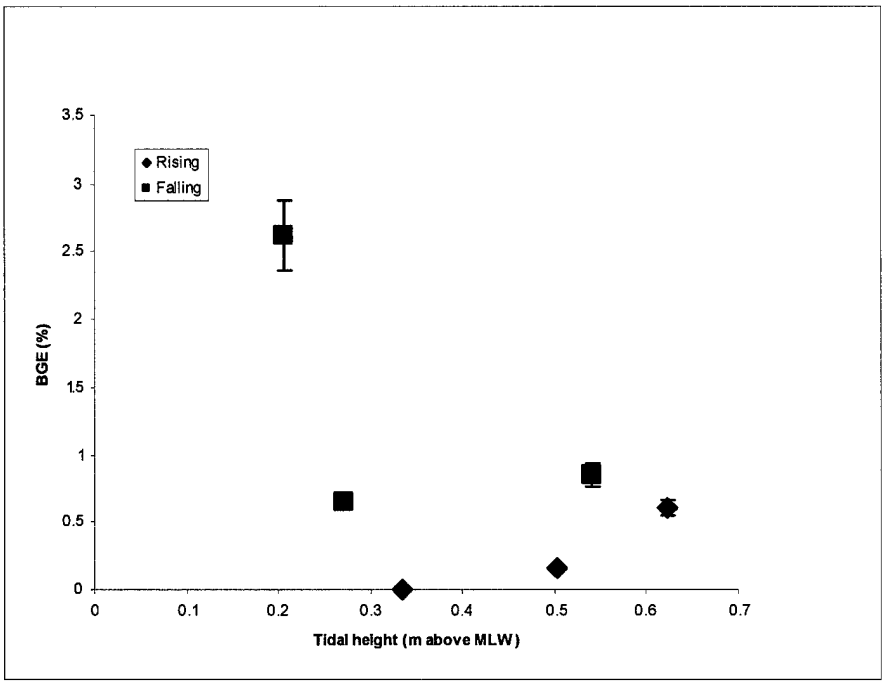

Fig. 37 — Fluoranthene BGE related to tidal height (July 1999)

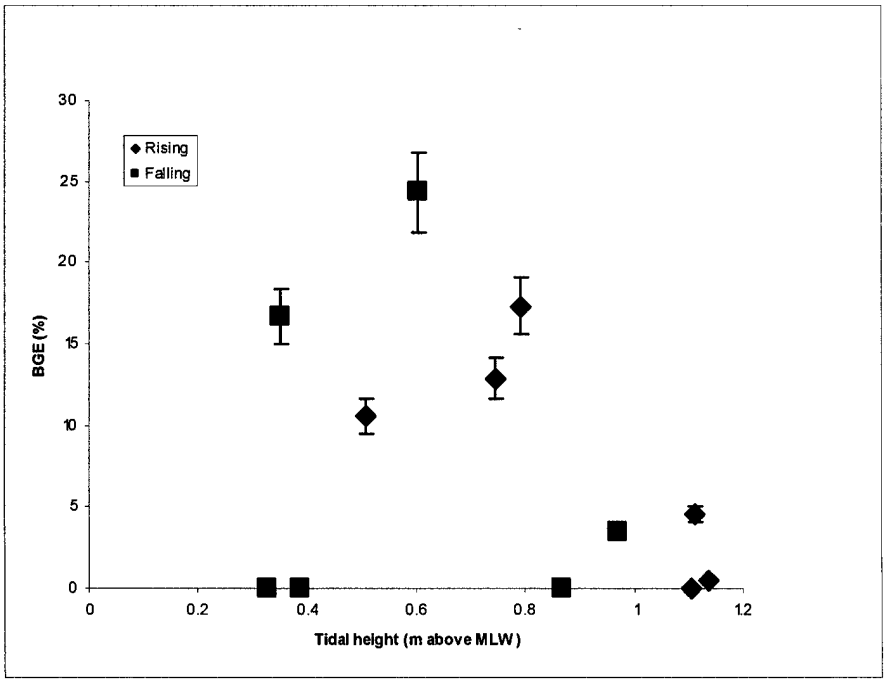

Fig. 38 — Fluoranthene BGE related to tidal height (October 1999)

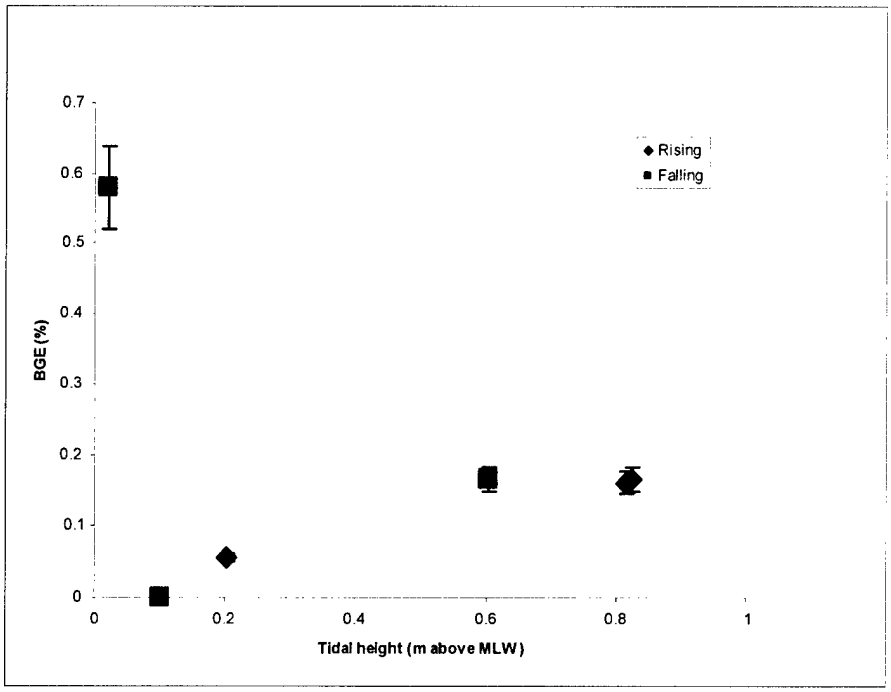

Fig. 39 — Fluoranthene BGE related to tidal height (March 2000)

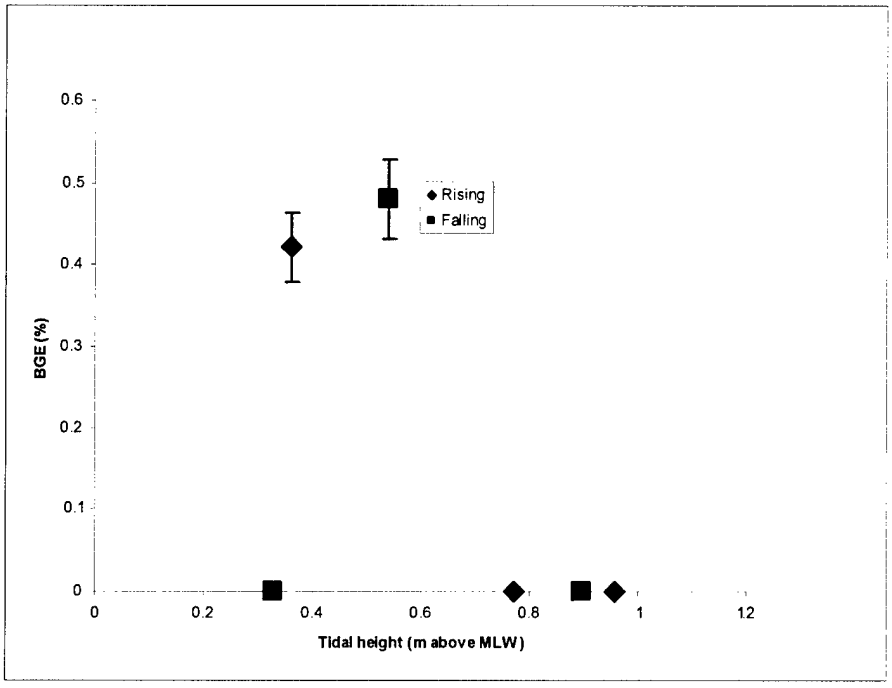

Fig. 40 — Fluoranthene BGE related to tidal height (September 2000)

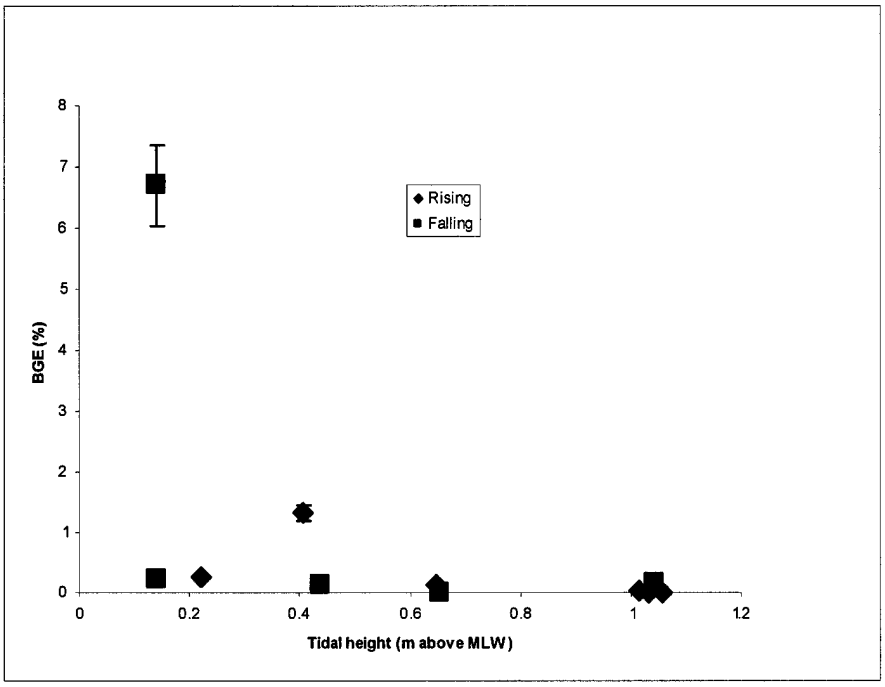

Fig. 41 — Fluoranthene BGE related to tidal height (July 2001)

Fluoranthene mineralization was generally higher in the groundwater than in the pierwater except during the rain event at the second and third time points (Fig. 42). This is opposite the trend that we saw with phenanthrene mineralization. Though fluoranthene mineralization did not show a trend with increasing dissolved oxygen concentrations, the highest rate did occur at the highest percent DO (Fig. 43).

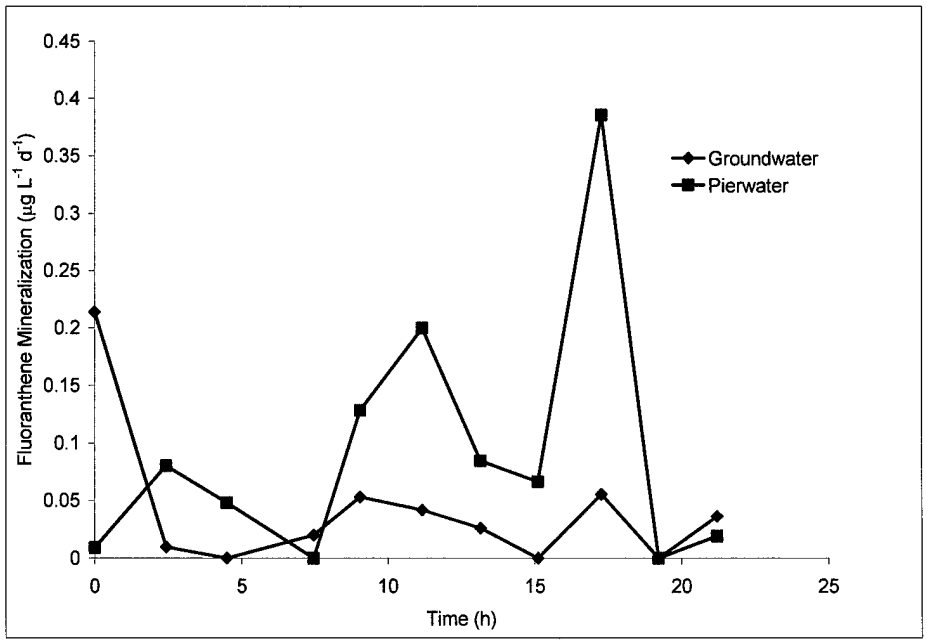

Fig. 42 — Fluoranthene mineralization was generally higher in pierwater than in groundwater over the course of the experiment.

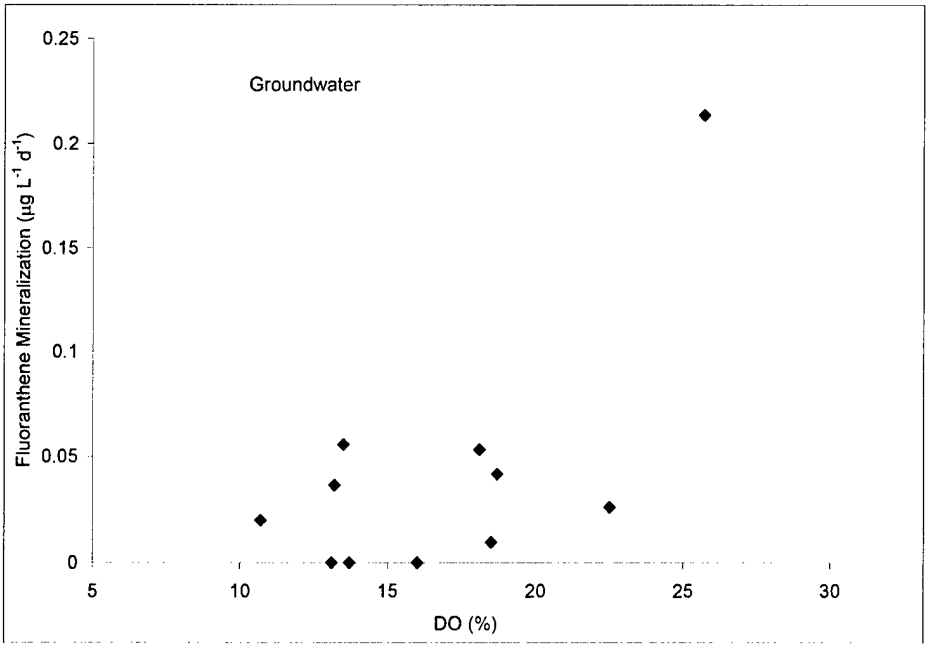

Fig. 43 — The highest fluoranthene mineralization occurred at the highest DO concentration measured over the course of the experiment

CONCLUSIONS

Based on our analyses to date, we were able to determine the rates of groundwater bacterial production and PAH biodegradation over the course of tidal cycles during several seasonal samplings. The following conclusions can be made:

- There was no significant amount of dissolved PAHs found in groundwater during any of the seasonal sampling events.
- Relatively low and invariant salinity in groundwater suggests reduced mixing between groundwater and Elizabeth River water in the subsurface.
- Submerged sediments off the pier directly adjacent to the monitoring well showed significant concentrations of PAH, indicating little direct coupling between soluble groundwater fuel hydrocarbons and those found in sediments.
- PAH mineralization was significant with nonpyrogenic PAH degraded most rapidly. This indicates indigenous microbial assemblages are adapted to degrade on-site fuel hydrocarbons.
- Phenanthrene mineralization was more related to salinity (groundwater) and fluoranthene mineralization was more related to DO concentration (pierwater) suggesting that different bacterial assemblages are responsible.
- Groundwater bacterial production was highest during warmer seasons mimicking trends traditionally seen in adjacent estuarine water.
- BGE was highest during colder samplings indicating fuel hydrocarbons are a more important source of carbon to the indigenous bacteria when cooler temperatures prevail.
- There was insufficient tidal recharge to elevate groundwater DO concentrations above hypoxia. There was no apparent stimulation of bacterial production or PAH mineralization within the DO range measured during this experiment.
- Stable carbon isotope signatures measured are not in the range of new petroleum stored in the base tanks.

To better understand the processes described above and to determine the seasonal dynamics of chemical contaminants and biological activities, the following studies are suggested:

- A survey of wells should be conducted to find areas with the highest tidal influence as measured by changes in wellwater heights. Also large changes in salinity could be used to identify areas where estuarine and groundwater are mixed.
- Wells near the area of highest recharge should be intensively sampled during tidal cycles. These are likely areas where significant changes in DO concentrations will be found. Furthermore, these are the most likely areas where offsite migration of dissolved fuel components occurs.
- Chemical and stable carbon isotope fingerprinting should be conducted on groundwater from high recharge sites and the sediments in the Chesapeake directly adjacent to determine if soluble fuel components are contaminating Navy sediments.
- Areas where high recharge occurs should be sampled for biological activity and fuel biodegradation rates. These rates coupled with hydrocarbon mass quantities in sediments should provide a means to assess the impact of groundwater fuel contamination to adjacent environments.
- Natural abundance radiocarbon isotope analysis of inorganic carbon in the groundwater and vadose zone may be used as an in situ confirmation of bacterial processes measured with the radiotracer addition experiments.

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Appendix

SUMMARY OF ANALYTICAL METHOD

SEMIVOLATILES ANALYSIS

Water

PAHs are extracted from water samples using a modification of a recent literature method (Boyd 1994). The method is briefly summarized as follows: Precondition a C_{18} Prep-Sep solid phase extraction (SPE) column with a methanol, then MilliQ water rinse. Add 100 µL surrogate standard to 1 L sample. Pass a 500 mL water sample (NaOH preserved) through the SPE column using vacuum (~50 mm Hg). Dry column for 3-4 days in a desiccant chamber. Elute dry column with two 500 µL aliquots of methylene chloride. Analyze 2 µL of eluant by GC/MS.

Sediment

PAHs are extracted from soil and analyzed using a modification of EPA SW 8270 (Fisher et al. 1997) as follows: Centrifuge sediment samples at 7,000 × g for 5 minutes. Pour off excess water. Separate out 10-15 g (wet wt.) sediment sample (depending on water content). Add 100 µL of surrogate standard mix to sediment sample. Weigh sample. Remove approximately 1 g (wet wt.) and transfer to a pre-tared aluminum weighing dish. Dry this subsample at 100 °C overnight and weigh for dry weight calculations. Add pre-baked diatomacious earth stepwise to sediment sample until dry. Transfer sediment to stainless steel Accelerated Solvent Extraction (ASE) cell[4]. Extract sediment for 20 min (100 °C / 2400 psi) with 1:1 acetone:methanol. Dry extract under a gentle stream of purified N_2 gas until volume is reduced to 250 µL. Analyze 2 µL by GC/MS.

BACTERIAL PRODUCTIVITY

^{3}H-Leucine is added to water or sediment and incubated to determine the amount of protein synthesis occurring within the bacterial population. Bacterial protein synthesis (via ^{3}H-leucine incorporation) is proportional to the increase of population biomass as described by Smith et al. (1992), as follows: Add a 1.0 mL water sample, or 50 µL sediment sample to a microfuge tube. Prepare tubes in triplicate with one killed control. Add 20 nM (final concentration) ^{3}H-leucine (ca. 1×10^6 DPM) to each tube and incubate at *in situ* temperature for 1 h. Add 100% (w/v) trichloroacetic acid to a final concentration of 5% (w/v) into each tube (to lyse bacteria and precipitate proteins). Perform this step at t = 0 for the killed controls. Centrifuge tubes for 7 minutes at 10,000 × g. Aspirate the supernatant (1.1 mL of waste) and wash the protein pellet with 1 mL of 5% (w/v) TCA, vortex and recentrifuge (7 min, 10,000 × g). Aspirate the supernatant (1.0 mL waste) and wash the pellet with 0.5 mL of 80% (v/v) ethanol, vortex and recentrifuge (7 min, 10,000 × g). Aspirate the supernatant (0.5 mL of waste) and add 0.5 mL of scintillation cocktail, vortex, and radioassay for ^{3}H. Relate protein synthesis to bacterial productivity, using the following equations:

[4] Dionex Corporation, ASE 2000, 33 mL cell.

$$\text{g C mL}^{-1}\text{ h}^{-1} = \left(\frac{\text{DPM}_{sample}}{\text{hr mL}}\right)\left(\frac{\text{mol leucine}}{\text{Y DPM}}\right)\left(\frac{2\ X\ 10^{17}\ \text{Cells produced}}{\text{mol leucine}}\right);$$

$$\text{where Y} = \left(\text{specific activity}\right)\left(\frac{2.22\ X\ 10^{6}\ \text{DPM}}{\text{Ci}}\right)$$

CONTAMINANT MINERALIZATION

$^{14}CO_2$ production was monitored by using the following method. This method was used for all sediment and water samples as follows: Transfer triplicate 1 mL water or 1 mL sediment subsamples to 150 × 16 mm test tubes. Do likewise to one control. To the set of triplicates and control, suspend filter paper (soaked with 100 µL 1 N NaOH). This acts as a CO_2 trap. Add 1 mL 2 N H_2SO_4 to the control tube. Add ^{14}C-labeled substrate (currently available for use: benzene, toluene, naphthalene, fluoranthene, and phenanthrene) to a final concentration of about 500 ng g^{-1} (depending on supplied specific activity). Incubate samples at collected water temperature for 12 to 24 h. To incubated samples, add 1 mL 2 N H_2SO_4. Place tubes on rotary shaker for 4 to 6 h (preferably overnight). Remove and transfer NaOH soaked filter papers to 2 mL centrifuge tubes. Amend all scintillation vials with 1 mL scintillation cocktail and radioassay. Prepare triplicate tubes from one sample site with addition of 0.05 µCi $NaH^{14}CO_3$ to determine $^{14}CO_2$ recovery efficiency. Treat similarly from steps 6-8. Relate respiration to contaminant removal using the following equation:

$$\text{Mineralization}\ \left(\frac{\text{g}}{\text{L h}}\right) = \left(\frac{\text{DPM}_{sample}}{\text{L h}}\right)\left(\frac{\text{mol substrate}}{\text{Y DPM}}\right)\left(\frac{\left(\frac{\text{g substrate}}{\text{\# of labeled carbons}}\right)}{\text{mol substrate}}\right);$$

$$\text{where Y} = \left(\text{specific activity}\right)\left(\frac{2.22\ X\ 10^{6}\ \text{DPM}}{\text{Ci}}\right)$$

PREPARATION OF SURROGATE STANDARDS FOR PAH ANALYSIS

PAH surrogate standards were prepared by making up a stock solution of 2-fluorobiphenyl and *p*-terphenyl-d_{14} at 100 mg L^{-1} each in Optima grade methanol.

GC/MS OPERATING CONDITIONS

A Hewlett-Packard 6890 GC coupled to a 5973 MS was used for PAH analysis. A 60 m 5% phenyl 95% methyl (SPB-5) 0.250 µm ID capillary column was used. Samples were injected by means of an autosampler into a splitless mode inlet maintained at 250 °C. Pressure pulse programming was used to increase inlet pressure from 16 to 25 psi prior to the septum purge at 2 min into the run. Overall column flow was 1.0 mL min^{-1}. Helium was the carrier gas. The initial column temperature was 40 °C. The temperature was ramped as follows: 1) at 1 min run-time, ramp 4.0 °C min^{-1} to 180 °C, hold 5 min. 2) ramp 4.0 °C min^{-1} to 220, hold 5 min. 3) ramp 4.0 °C min^{-1} to 280 °C, hold 5 min. 4) ramp 4.0 °C min^{-1} to 300 °C, hold 10 min. The distal column end empties directly in the source area of the 5973 MS. The MS was tuned with perfluortributlyamine (PFTBA). The MS temperature was set to 106 °C; the source was set to 230 °C.

QUALITY CONTROL SAMPLING

Various QC samples were collected during field operations to include field blanks and trip blanks.

Field Blanks

Field blanks were collected by rinsing sampling equipment with MilliQ water and collecting the rinse water. The rinse was analyzed for organics in a similar manner to water column samples. This analysis defines the contribution of equipment-borne contaminants to the samples.

Trip Blanks

A trip blank was prepared in the laboratory for shipment to the sample site. It consisted of a centrifuge tube filled with precombusted Ottawa sand. It was kept with the sampling equipment and handled the same as regular samples. The trip blank served to elucidate the contribution of outside contamination to samples collected, shipped, and stored before analysis.